

# Abstracts of Scientific Presentations

## 2009 AALAS National Meeting

### Denver, Colorado

#### Platform Sessions

##### PS1 Intestinal Flora Promotes Gastritis and Premalignancy in *Helicobacter pylori*-Infected INS-GAS Mice

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Transgenic (Tg(Ins1-GAS)1Sbr) (INS-GAS) mice develop hypergastrinemia and thus are predisposed to gastric hyperplasia, atrophy, and neoplasia. Male INS-GAS mice infected with *Helicobacter pylori* develop gastric carcinomas by 6 to 7 mo postinfection (MPI). The progression of gastritis to gastric atrophy may enable colonization of the stomach with intestinal flora, which may then promote the progression to gastrointestinal neoplasia (GIN). To examine this hypothesis, 20 male and 27 female germfree INS-GAS mice were orally dosed with  $1 \times 10^8$  colony forming units of *H. pylori* SS1 at 6 wk of age and were compared to germfree control mice for histopathology, serology, and serum cytokines at 5, 7, 9, and 11 MPI. Infection was confirmed with isolation of *H. pylori* from gastric mucosa. At all time points, infected mice demonstrated more severe pathology and proinflammatory serum cytokine and IgG isotype responses than uninfected controls ( $P < 0.05$ ). Infected males developed more severe gastric pathology than infected females and higher mRNA expression levels of IL-1 $\alpha$ , IL-12 (both  $P < 0.05$ ), and TNF- $\alpha$  ( $P < 0.01$ ). Infected females also developed higher antiinflammatory IgG1 responses by 11 MPI ( $P = 0.02$ ). Although these findings were consistent with previous reports, *H. pylori*-monoinfected INS-GAS mice did not develop significant gastric pathology until 9 MPI, at which time 4 of 11 mice had developed GIN. These findings contrast to a previous report that SPF *H. pylori*-infected INS-GAS mice developed severe gastric neoplasia by 6 MPI, with 10 of 10 developing GIN. These findings suggest that gastric atrophy, with associated elevated gastric pH, in the INS-GAS mouse creates a colonization niche for intestinal flora to further promote GIN. Thus, the presence of intestinal microflora appears to be a critical factor in the progression and severity of gastric pathology in the INS-GAS mouse when infected with *H. pylori*.

##### PS2 A Coinfection Model in Mongolian Gerbils Infected with *Brugia pahangi* and *Helicobacter pylori*

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*Helicobacter pylori*, a gram-negative bacterium that infects the stomach, is a major cause of gastritis and peptic ulcers and may lead to cancer. In African human populations with high rates of *H. pylori* infection and low gastric cancer rates, individuals are commonly coinfecting with nematodes, including *Wuchereria bancrofti*. The Mongolian gerbil is a suitable model because when infected with *H. pylori* it develops marked quantifiable gastric lesions and is also a permissive host for *Brugia pahangi*, a filarid nematode. The goal of this study was to evaluate gastric pathology and cytokine responses in gerbils infected with *H. pylori* or *B. pahangi* versus those coinfecting with *H. pylori* and *B. pahangi*, which may be helpful in explaining why coinfecting humans are at a decreased risk for developing *H. pylori* associated gastric cancer. Gastric pathology was scored for inflammation, epithelial defects, hyperplasia, and dysplasia, and the cumulative scores were represented as gastric histologic activity index (HAI). At 21 wk post-*H. pylori* infection (WPHpI), significantly ( $P < 0.05$ ) increased dysplasia and increased HAI were observed in the *H. pylori*-only group as compared to the dually infected group. At this time point, the worm burden was inversely correlated with gastric pathology ( $P < 0.05$ ). At 42 WPHpI, no significant differences in gastric

histopathology scores were detected between the mono- and dual-infected groups and there were no differences in parasite burden. Cytokine profiles were assessed and at 21 WPHpI, all cytokines (IFN- $\gamma$ , IL-1 $\beta$ , CXCL1, IL-4, IL-10) analyzed were upregulated, though no statistically significant changes were seen between groups. Although all 5 cytokines analyzed were downregulated in the *B. pahangi*-only group and upregulated in the other experimental groups, at 42 WPHpI cytokine analysis again showed no significant difference between the groups. This is the first study using a Mongolian gerbil coinfection model to ascertain the interplay between *H. pylori*-induced gastric pathology and heterologous immune modulation due to parasitic infections.

##### PS3 Seroconversion of Syrian Hamsters to a Novel *Helicobacter* Species Isolated from a Hamster Liver

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Syrian hamsters (*Mesocricetus auratus*) have historically been used experimentally to study liver carcinogenesis associated with carcinogen administration and liver fluke infection. Research colony hamsters have been shown to be infected with multiple species of *Helicobacter* with associated gastritis and inflammatory bowel disease. Older hamsters commonly have idiopathic liver lesions, which in some cases progress to cholangiocarcinoma. We screened 10 retired breeding hamsters from 2 commercial sources to determine if hepatic lesions may be associated with *Helicobacter* infection as has been documented in select strains of inbred mice. Five female hamsters from 1 source were *Helicobacter* free in both the cecum and liver using genus-specific PCR for a 1.2 kb fragment of the 16srRNA. Five male hamsters from the second source were all PCR positive in the cecum, and 1 of 5 livers was PCR and culture positive. The *Helicobacter* isolate from the liver was determined to be a novel urease negative *Helicobacter* species by 16srRNA analysis. Sera were tested by ELISA against outer membrane preparations of these *Helicobacter* isolates cultured from the liver and cecal samples. There was robust discrimination by ELISA using the liver isolate antigens between the *Helicobacter*-free hamsters from the first source and those demonstrated to be infected from the second source ( $P < 0.001$ ). Because liver histology demonstrated only minimal to mild periportal and lobular inflammation in hamsters from both sources, the serology data suggest that a controlled experimental infection with the novel liver isolate in *Helicobacter*-free hamsters from the first source would further define the role of *Helicobacter* as a cause of liver lesions commonly noted in older hamsters.

##### PS4 The Effect of *Helicobacter hepaticus* Infection on Herpes Simplex Virus Type 1-Specific Immune Responses and Characteristics of Dendritic Cells

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Contamination with *Helicobacter hepaticus* is very common in research mouse colonies; however, little is known about how this persistent infection affects immunologic research conducted in *H. hepaticus*-infected mice. We sought to determine if herpes simplex virus type-1 (HSV-1)-specific immune responses and characteristics of dendritic cells (DCs) are altered by *H. hepaticus* infection. We inoculated 3-wk-old male C57BL/6 mice with 10<sup>9</sup> cfu of *H. hepaticus* or saline by oral gavage and performed experiments 1 mo later. Each experiment was conducted twice. We measured virus-specific antibody by plaque assay and CD8+ T cell-mediated immune responses by flow cytometry in *H. hepaticus*-infected and noninfected mice 1 wk after intranasal inoculation with HSV-1 ( $n = 10$  per group). The effect of *H. hepaticus*

on the HSV-1-specific antibody in the serum and T cell-mediated immune responses in superficial cervical and tracheobronchial lymph nodes (LNs) did not reach the level of statistical significance. In separate experiments, we used flow cytometry to compare the expression of maturation-associated surface markers CD40, CD80, CD86, and MHC class II and proinflammatory cytokines IL-12p40 and TNF- $\alpha$  by DCs from the spleen and colic LNs of *H. hepaticus*-infected and noninfected mice ( $n = 15$  per group). There was a decreased surface expression of CD40, CD86, and MHC class II and a decreased percentage of IL-12p40 and TNF- $\alpha$ -producing DCs from colic LNs of *H. hepaticus*-infected mice. In contrast, *H. hepaticus* infection did not inhibit the splenic-derived DCs. In fact, there was an increased expression of CD40, CD80, CD86, and MHC class II on splenic-derived DCs from *H. hepaticus*-infected mice following in vitro lipopolysaccharide stimulation. These results indicate that *H. hepaticus* infection can influence the results of immunologic assays in mice and support an argument for the use of *H. hepaticus*-free mice in immunologic research.

#### PS5 Murine Norovirus-4 Infection Does Not Alter Diet-Induced Obesity and Insulin Resistance in C57BL/6 Mice and LDLR-/- Mice

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Murine norovirus (MNV), endemic in many SPF mouse colonies across North America, poses significant potential interference with mouse models of human disease. Presence or absence of MNV in mouse colonies could help explain phenotypic variabilities in mouse models across institutions. We wished to determine whether MNV could influence the development of obesity, insulin resistance, and associated pathologic sequelae in 2 widely used animal models of such disease, C57BL/6 mice and low-density lipoprotein receptor-deficient (LDLR-/-) mice. We hypothesized that MNV infection may accelerate and increase severity of obesity-associated phenotypes such as insulin resistance and atherosclerosis by changing macrophage activity or accumulation in adipose tissue. In the first study, C57BL/6 male mice were placed on a high-fat diet (approximately 60% fat) at 6 wk of age and continued on the diet for 10 wk. Mice were divided into 3 groups ( $n = 10$  per group); the first and second groups were gavaged with either vehicle or MNV-4 ( $5 \times 10^6$  pfu) at the start of the diet, and 4 wk after initiating the diet, the third group was infected with MNV-4. In the second study, 6-wk-old LDLR-/- male mice were fed a Surwit diet for 16 wk using the same MNV treatment regimen as in the first study ( $n = 12$  per treatment). All infected mice seroconverted and remained infected during the study (10 wk in C57BL/6 and 16 wk in LDLR-/- mice) as verified by fecal PCR. Mice in both studies were evaluated for food intake, body weight changes, insulin resistance by glucose and insulin tolerance tests, adiposity, serum lipids, and liver lipidosis. LDLR-/- mice were also evaluated for atherosclerosis using aortic sinus lesion analysis. No significant differences were noted between uninfected and MNV-infected mice with respect to obesity, insulin resistance, and atherosclerosis. Studies are underway evaluating the effects of MNV-4 infection in a more robust model of atherosclerosis, LDLR-/- mice fed a high-fat, high-cholesterol diet (Western).

#### PS6 Infection with Murine Norovirus-4 Does Not Alter Bacterial Triggered Colon Cancer in Smad3 Null Mice

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Murine norovirus (MNV) is a prevalent viral pathogen in mouse colonies and causes disease and mortality in severely immunocompromised mice. We have shown that infection with MNV-4 alters the disease phenotype and immune function in a *Helicobacter*-induced model of inflammatory bowel disease in FVB.129P2-Abcb1a<sup>tm1BorN7</sup> (mdr1a-/-) mice. To determine if a model of inflammation-associated colon cancer would also be altered by MNV infection, we assessed the effect of MNV on the time to development and incidence of *H. bilis*-induced colon cancer in 129-Smad3<sup>tm1Par</sup>/J(Smad3-/-) mice. Four separate studies were conducted in which 3- to 4-mo-old male and female Smad3-/- mice were treated with vehicle, MNV-4 alone ( $1$  to  $5 \times 10^7$ ), *H. bilis* alone ( $10^4$  to  $10^7$ ), or MNV followed by *H. bilis* 1 wk later ( $n = 8$  to 12 per group). Study 1 groups were MNV/*H. bilis* and *H. bilis*.

Study 2 groups were MNV/*H. bilis* and *H. bilis*. Study 3 groups were MNV, MNV/*H. bilis* and *H. bilis*. Study 4 groups were vehicle, MNV/*H. bilis* and *H. bilis*. Mice were necropsied at 17 to 32 wk after infection or if body condition deteriorated. All MNV- and *H. bilis*-infected mice remained infected (verified by fecal PCR), and MNV-infected mice seroconverted to MNV. Histopathologic analyses of Study 1 to 3 and gross necropsy results of Study 4 indicated no significant differences between mice coinfecting with MNV/*H. bilis* versus *H. bilis* alone, in survivability or development of colonic mucinous adenocarcinomas. No tumors developed in Smad3-/- mice given media or RAW cell lysate or MNV-4 alone. Additionally, since MNV can persistently infect macrophages, we isolated macrophages from bone marrow of Smad3-/- and Wt mice and infected them with MNV-4. After 48 h, RNA was extracted and assayed for cytokine expression by real-time PCR. There were no significant differences in IL-1 $\alpha$ , IL-1 $\beta$ , or IL-6 expression from MNV-infected macrophages derived from Smad3-/- or Wt mice. We conclude that infection with MNV-4 does not appreciably affect this model of inflammation-associated colon cancer.

#### PS7 Effect of Murine Norovirus Infection on Mouse Parvovirus Infection in BALB/c Mice

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Endemic infection with mouse parvovirus (MPV) remains a common problem in laboratory colonies. Diagnosis of MPV infection is complicated by the effects of mouse age and strain, as well as virus dose and strain on susceptibility to infection. The potential for an intestinal virus coinfection to affect susceptibility to MPV infection was investigated. Murine norovirus (MNV) is the most prevalent infectious agent in laboratory mice and causes a chronic subclinical infection in most strains of immunocompetent and immunocompromised mice. The effect of an MNV infection 1 wk prior to MPV infection on seroconversion to MPV, fecal shedding of MPV, quantity of MPV DNA in tissues, and transmission to sentinels was assessed in 6-wk-old BALB/c mice. All 5 mice inoculated with MNV and MPV were MPV seropositive at 1 wk post-MPV-inoculation (WPI), while only 20% of mice inoculated with MPV only were MPV seropositive at 1 WPI. MPV DNA was detected by PCR in pooled feces from all 12 cages of mice infected with MPV only at 1 WPI but not at 2 to 5 WPI. In contrast, MPV DNA was detected in pooled feces of all cages of mice coinfecting with MNV and MPV on 1, 2, and 3 WPI. A significant difference in transmission of MPV to SW mice housed in soiled cages at 1 and 2 WPI was not detected between the MPV only (15 of 20) and MNV/MPV (20 of 20) groups. Compared to mice infected with MPV only, mice infected with MNV and MPV had higher quantities of MPV DNA in the small intestines, mesenteric lymph nodes, and spleens at 1 WPI, and in the mesenteric lymph nodes and spleens at 2 WPI ( $P < 0.05$ ). MNV infection in BALB/c mice increased the quantity of MPV DNA in feces and tissues and the duration of shedding, increasing the ability to detect MPV directly in coinfecting mice, but did not affect transmission to soiled bedding sentinels.

#### PS8 Mouse Parvovirus Infection in BALB/c, C57BL/6, and Swiss Webster Mice

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Endemic infection with mouse parvovirus (MPV) remains a common problem in laboratory colonies, and diagnosis of MPV infection is complicated by many host and environmental factors. The effect of mouse genotype on seroconversion to MPV, fecal shedding of MPV, amounts of MPV DNA in tissues, and transmission to sentinels was assessed in 2 immunologically divergent inbred strains (BALB/c, C57BL/6) and an outbred strain (Swiss Webster; SW) of mice. All 5 SW and 5 BALB/c mice were MPV seropositive at 1 and 2 wk post inoculation (WPI), respectively; however, at 2 WPI C57BL/6 mice were still not seropositive. At 4 WPI 28 of 30 C57BL/6 mice were MPV seropositive. MPV DNA was detected by PCR of pooled feces from all 12 cages of BALB/c and C57BL/6 mice at 1 WPI, but not at later times. In C57BL/6 mice, low amounts (5 to 14 fg/ $\mu$ L) of MPV DNA were detected in the small intestines (SI) at 1 WPI, but were not detected in mesenteric lymph nodes (MLN), spleens, or colons. In BALB/c mice, low amounts of MPV DNA were detected in the SI (2 to 27 fg/ $\mu$ L) and colons (0

to 16 fg/ $\mu$ L), and higher amounts were detected in the MLN (261 to 1,519 fg/ $\mu$ L) at 1 WPI. In SW mice, MPV levels were significantly higher than in BALB/c or C57BL/6 mice at 1 WPI ( $P < 0.03$ ), with levels of 54 to 122 fg/ $\mu$ L in colons, 80 to 856 fg/ $\mu$ L in spleens, 1,190 to 6,890 fg/ $\mu$ L in SI, and 22,202 to 65,845 fg/ $\mu$ L in MLN. At 2 WPI, MPV DNA was detected in all tissues from SW mice, MLN only from BALB/c mice, and no tissues from C57BL/6 mice. Although MPV DNA levels in MLN of SW and BALB mice were considerably less at 2 compared to 1 WPI, inoculations of MLN from both times resulted in productive infections. Transmission of MPV using soiled bedding from cages of infected BALB/c and C57BL/6 mice differed at 2 WPI (47% versus 0%;  $P = 0.02$ ). These results indicate that MPV DNA levels in tissues are highest in SW, intermediate in BALB/c, and lowest in C57BL/6 mice. MPV DNA levels in the MLN of BALB/c and SW mice significantly exceeded those of the SI and feces whereas the inverse occurred in C57BL/6 mice. C57BL/6 mice also had the longest time to seroconversion and shortest window of transmission.

#### PS9 Diagnostic Strategies between Polytypic and Enteric Strains of Mouse Hepatitis Virus

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Polytypic strains of mouse hepatitis virus (MHV) include A59, JHM, S, 1, 2, and 3. Enteric strains, which are the most prevalent strains detected in the field today, include Y, D, and RI. We investigated diagnostic strategies for rapid detection of MHV outbreaks in laboratory mice using a combination of serology and PCR techniques. A polytypic A59 strain and an enteric Y strain were chosen to assess the differences in antibody response during seroconversion and to check the efficacy of MHV antigens to detect antibodies against both strains. Real time PCR (RT-PCR) was used to determine the differences in tissue tropism between 2 strains. Four to 6-wk-old female CD-1 mice were inoculated intraperitoneally with A59, and intranasally and orally with Y. Sera and tissues were collected from inoculated mice at 0, 7, 10, 14, 21, 28, and 35 d post inoculation (DPI). Tissues for RT PCR included mesenteric lymph nodes (MLN), liver, and feces. MHV seroconversion was assayed by MFIA using conventional (A59 and S) and recombinant antigens, and by ELISA and IFA using conventional A59 antigen. All antigens used in MFIA detected seroconversion at the same DPI as did ELISA and IFA. Seroconversion for A59 inoculated mice was detected at 7 DPI and comparatively for Y was delayed to 10 DPI, although RT-PCR results suggested active virus replication at 7 DPI for both strains. Both viruses were detectable in the MLN 7 to 35 DPI (1,000 copies/mg) and in feces for Y at 7 to 35 PI (100 copies/mg). Fecal shedding of A59 was detectable at 7 and 10 DPI. A59 virus was detected in the liver at 7 to 14 DPI, and Y was never detected in the liver. In summary, MHV antigens efficiently detected the seroconversion early in experimentally infected mice. MLN is a good tissue for the detection of MHV infection by PCR for both viruses. Feces are a good noninvasive alternate for Y and most likely other enteric MHV strains, but may be limiting for A59 and other strains that do not replicate in the gut. A combination of serology, MLN, and/or fecal PCR will improve the likelihood of early detection of an MHV outbreak.

#### PS10 Occult Cytomegalovirus in Vivarium-Housed Mice

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We have recently shown that murine cytomegalovirus (MCMV) can influence murine transplant allograft acceptance. During these studies, results suggested that vivarium-housed control mice can acquire occult MCMV. The purpose of this investigation was to confirm occult MCMV transmission and determine the kinetics and vector of transmission. We began by evaluating naive mice arriving from our commercial vendor for MCMV by commercial testing and by nested PCR for MCMV DNA. Upon arrival, mice were negative for MCMV both by commercial testing and by nested PCR. Mice housed in our vivarium were tested for MCMV at various intervals after arrival to determine the kinetics of occult MCMV infection. All mice became positive for MCMV DNA 30 to 60 d after arrival but remained negative for MCMV by commercial testing. Despite harboring MCMV DNA, these mice had minimal MCMV reactive antibody even after 1 y of housing. To

confirm MCMV we sequenced PCR products for several genes and showed more than 99% homology to MCMV. Because our microisolation lids should minimize cage to cage transmission, we considered that mouse chow might be a source of virus and thus evaluated chow for MCMV DNA. Although mouse chow contains detectable MCMV DNA, the DNA sequences are different from those seen in occult infections. Further sequence analyses show that the occult infections are from a laboratory strain of MCMV, suggesting horizontal transmission, but the vector remains unclear. These data confirm that vivarium-housed mice can develop occult MCMV that is missed by currently available commercial testing. Given our experience with MCMV influencing transplantation outcomes in mice, unexpected outcomes in immunology experiments should raise suspicion of occult MCMV and should prompt evaluation using our highly sensitive PCR-based methodology.

#### PS11 Stereotypic Behaviors in Singly Housed Rhesus Macaques Are Significantly Reduced during Aquarium Viewing

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Stereotypic behavior (SB) is a common problem in laboratory nonhuman primates, particularly those that are individually housed. Cage enrichments or toys are frequently used in an effort to reduce SB. Typically, however, they are only effective if performance of the SB is incompatible with toy manipulation. Although fish tank viewing does not involve manipulation, there are several reasons to think it might be effective in reducing SB. For example, with humans, fish tanks are used for their calming effect under stressful circumstances such as in dentists' offices. Further, in common with other effective enrichments, fish observation is a component of a natural behavior (fishing) in some macaques. This study investigated the effect of viewing an aquarium containing live fish on the frequency of preexisting SB in 8 individually housed rhesus macaques: 3 with spinning, 3 with swinging, and 2 with pacing. Baseline (video only) and control (video plus aquarium containing water) SB were established by placing the apparatus in the room and recording for 1 h per day for 5 d. Baseline and control SB were not significantly different from each other. During test conditions, the aquarium was placed in front of the cage for 1 h daily (Monday through Friday) for 4 wk and the macaques videotaped during the Monday and Friday sessions. The number of SB under test conditions was significantly reduced (spinning 44%, swinging 41%, and pacing 50%) compared with control (paired t test,  $P = 0.00003$ ). There was no habituation to the fish tank during the 4-wk exposure. We suggest that viewing aquaria containing fish can reduce SB in rhesus macaques and should be considered as a component of enrichment programs for nonhuman primates with SB.

#### PS12 Risk-Based Assessment of Animal Facility Operations

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We approach animal facility operations as a business model through a financial auditor's perspective by defining how internal controls and risk assessment concepts affect operations throughout animal facilities. This method applies audit tests to significant animal facility operations based on risk assessments. Two questions must be answered when considering risk: 1) What is the importance of the operation to the animal facility, and 2) how probable is it that inconsistencies and irregularities will occur? For example, if your assessment of room maintenance demonstrates the facility has good business practices in place, you might determine that risk is both low in importance and probability, and your audit test sample of room maintenance data may be minimal. However, if the opposite is true (risk is high), you would need to test more room maintenance data for propriety. By sampling data after your risk assessment is completed, your staff can determine if certain operations (financial or nonfinancial) of the animal care facility need improvement, are meeting the organization's goals, are up to generally accepted standards, or are complying with appropriate laws and regulations. When all of these independent tests are put together, you can form an audit program that can be used to determine if the organization has made progress towards becoming more efficient and effective. Results from an integrated audit program can help managers focus on areas where they should expend more effort in gaining better efficiency or effectiveness, or install stronger internal controls.



### **PS13 Animal Centered Planning Using Essential Lifestyle Planning Tools**

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Best practices in the animal research environment include a plan for quality care of the animals, which is implemented by a caring and cohesive team of experts. An excellent method of developing animal plans of care is to use essential lifestyle planning (ELP), a person-centered planning tool that is equally effective for animals as it is for humans, given that it was originally created for people who do not necessarily use words to communicate. A properly developed ELP will establish a perfect balance between what is important to the animal (things they like and enjoy) and what is important for the animal (things that are needed to keep them healthy and safe), and will rely upon collective observation and documentation by all team members as animals use behavior to communicate. ELPs are developed in partnership, acknowledging that no single person has all the answers and the contribution of each team member is valuable, enriching, and validating to the plan. The ELP is presented, reviewed, and updated at regular team meetings using current observational data. The plan is linked to and enhances standard operating procedures as it includes specific details regarding the animals' likes and dislikes and how they go about communicating this information. Since ELPs include a "to-do list," goals, and an action plan, there is no question as to what needs to be done. When the team has completed and agreed upon the plan a suitable template is selected and the plan is shared and preserved electronically. Essential lifestyle planning keeps the animals at the center of the plan, thus allowing the team to remain focused, objective, and proactive in their pursuit of quality care for animals in research.

### **PS14 Web-Based Solutions for Management of Animal Resources**

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Effective communication between investigators and animal care staff is crucial to support research objectives. As such, dealing with frequent unique requests for services, space, and equipment is burdened with miscommunication. Methods typically rely on paper forms, calendars, emails, phone calls, and informal conversations. In many instances, these methods are inefficient, redundant, and unreliable and ultimately lead to confusion and delay. Purchasing or recreating transgenic mice lines that exist within the institute also waste time, energy, and money. To address these issues, we integrated software and database solutions into our departmental website accessed via a secured intranet to enable paperless, around-the-clock submission and tracking. We used a ticketing system that allows researchers to review and monitor service requests in real time, providing updates as work is completed. We designed a scheduling system to aid investigators in advanced planning with space and equipment availability and reservation. We created a searchable mouse repository of custom transgenic mice to maximize awareness of these valuable resources. By implementing these web-based solutions, we have streamlined efficiency in rendering services and strengthened our collaboration with research laboratories. The ticketing system has enhanced customer service by tracking activity and ensuring task completion. The scheduler has helped to provide equitable access to shared resources, especially considering the unpredictable availability of academic researchers. The repository database of custom transgenic mice is shared by institutional members and created new collaborations and research opportunities, while saving vital resources related to acquiring novel mouse lines. Feedback from user surveys concluded that these web-based solutions were well received and streamlined communication because of their simplicity and convenience. To ensure the success of these management tools, it is vital to involve end users in beta testing and provide user training. We also recommended having dedicated personnel to update, monitor, and maintain these systems.

### **PS15 Bioinformatic Analysis of Laboratory Animal Models Used in Vaccine Research and Development**

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Laboratory animal models have been widely used for vaccine research and development. In order to achieve the 3Rs of laboratory animal models used

in scientific research, the United States Department of Health and Human Services (HHS) and Food and Drug Administration (FDA) have instituted the Animal Efficacy Rule (also known as the "2 animal rule"). This mandate for development and approval of human therapeutics (for example, vaccines) requires demonstration of a "reasonably well-understood mechanism" and the use of at least 2 different animal species to demonstrate "response[s] predictive for humans." In order to better organize and study the plethora of animal models for vaccine research, we have developed a web-based vaccine research animal model database (Vaxmod) as part of the previously established Vaccine Investigation and OnLine Information Network. To date, Vaxmod includes various models from 15 different animal groups including rodents, rabbits, ferrets, primates, birds, and ungulates. These models have been used to study more than 300 vaccines or vaccine candidates for over 40 pathogens, including those significant to both humans and animals. All data stored in the Vaxmod database is publically available for web queries and analyses. Vaxmod uses our Vaccine Ontology (VO) system, a controlled vocabulary of vaccine-related terms and relations. The use of VO allows more efficient analysis of vaccine animal research models and makes possible the integration of this data with relevant data from other biologic databases. Two case studies will be presented, comparing and analyzing animal models used in the study of the tuberculosis vaccine BCG and brucellosis vaccine RB51. It is our hope that these bioinformatics approaches will increase understanding of laboratory animal models in vaccine-related studies, thus benefitting the research community, the veterinary and husbandry community, and the animals themselves.

### **PS16 Raising the Bar on Training**

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When job-specific skill training fails to eliminate performance gaps, poor communication, and poor staff morale, a staff development training plan might be the answer. Increasingly, institutions are recognizing that staff or professional skills development is the winning difference. The ability to feel, think, and act appropriately in any given situation that might arise in the animal facility is the hallmark of professionalism. Effective communication, problem solving, and improved customer care are just a few of the professional skills that can transform an animal care staff from good to great. This presentation will demonstrate that how we feel, think, and act has a direct correlation on our ability to communicate effectively, solve problems, make good decisions, and provide customer service. This presentation is designed for directors, managers, and supervisors who need to be able to recognize when training needs go beyond traditional training as well as provide them with the resources and tools to implement a culture of professionalism.

### **PS17 New Directions in Cage Processing Automation: Expanding Beyond the Dump Station**

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Much has been learned about the use of robotics in animal facilities during the last decade, and today almost every new facility is at least considering some type of automation due to its ability to improve the productivity and safety of cage wash operations. Once it was shown that large-scale, new facilities could be automated successfully, the focus turned to its implementation in existing and smaller scale operations. But dumping cages and placing them on a belt is not the only operation in the cage wash area that creates ergonomic and productivity impediments. In order to reduce cross-contamination and allergen exposure, many institutions opt to transport their cages as complete microisolation sets. This practice means that cages must be disassembled prior to washing—a process exposing the operator to allergens and repetitive motion injuries. The author manages a 600 m<sup>2</sup> mouse facility housing 5,000 ventilated cages. A complete cage change (bottom, wire, and top) is performed every 14 d. Cages are transported as complete setups to the cage wash, where they are manually disassembled. Bedding is dumped in a disposal station, and components are placed onto a wash rack and processed in a rack washer. Clean components are reassembled, supplied with bedding and feed, and sterilized. A robotic system was developed for the dirty side of the facility to automate the process of removing cages from the transport rack, disassembling them, placing wires and tops on a

wash rack, dumping the cage bottoms, and placing them on the wash rack. As part of the process, a new logistic transport unit was designed to work with the system so cages can be loaded onto transport trolleys in the holding room, brought to cage wash, and loaded directly into the automation cell without any additional manipulation. The number of cages sterilized per cycle increased from 160 to 200 with the use of the redesigned trolleys, improving by 25% the autoclave throughput and reducing energy consumption. To the best of the author's knowledge, this is the first time automation has been used not only to dump cages and stage them for washing, but to also include the operations of disassembling the cages.

### **PS18 Failure of Quarantine Bedding Sentinels to Detect *Helicobacter*, *Pasteurella pneumotropica*, and Murine Norovirus**

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Quarantine programs commonly use bedding sentinels to prevent the introduction of infectious agents into a rodent research colony. Sentinels are analyzed by serology or isolation techniques post exposure to detect bacteria and viruses. Challenges associated with the use of bedding sentinels for routine health monitoring of research facilities have been reported and observed. Limited information is available regarding bedding transfer success rates for most rodent agents. To investigate the efficiency of infectious agent transmission in a bedding sentinel quarantine program, a PCR microarray was used to test pooled feces collected directly from quarantined mice for infectious agents. Results were compared to those obtained by using standard bedding sentinel practices. Sentinels received dirty bedding from quarantine animal shipping crates and from cages during weekly cage changing. At 3 wk post exposure (PE), sentinel mice were bled and sera was evaluated via serology. Fecal samples were submitted for *Helicobacter* PCR testing. At 8 wk PE, sentinel mice were submitted for bacteriology and serology. The PCR array detected *Helicobacter* in 6 of the 9 quarantine groups, *Pasteurella pneumotropica* in 5 groups, and MNV in 5 groups. Neither *Helicobacter* nor *P. pneumotropica* was detected in any of the bedding sentinel mice. Murine norovirus (MNV) was undetected at 3 wk PE in sentinels representing 4 of the 5 quarantine groups that had been determined to be MNV PCR-positive. Two additional groups had seroconverted by 8 wk PE; however, 2 groups remained negative by serological testing. The bedding sentinel quarantine program failed to detect 3 relevant infectious agents that were detected by the PCR array. Direct testing of quarantine mice by PCR microarray may provide a more sensitive and efficient process to detect infectious agents during quarantine and could also serve to reduce the quarantine period.

### **PS19 Automation of Rodent Fasting**

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Fasting animals for use in a study is a standard practice in the laboratory animal industry. At times requests are made to fast animals outside the normal business hours, when little to no staff are available in the facility. Even when prior arrangements are made, scheduling issues often arise depending on the time of year and staff availability. Recently, certain projects in our facility required fasting to occur between 2200 and 0600 for both immunocompromised and syngeneic rodents. Therefore, it was necessary to automate the removal of access to feed in order to avoid having the staff manually having to pull the feed from the cages late at night. Since the request came from various groups, a universal design to fit all cage types was desired. We contacted our onsite automation engineering group to design a system that would automate this task in an accurate and dependable manner. The challenge was to design a device that would be functional and user-friendly without affecting the integrity of the primary enclosures (static microisolation cages). A food hopper was fabricated to rest in the wire bar lid, allowing the animals to have access to the food; the hopper was then attached to an air actuated piston that extended and caused the food to be lifted away (by 0.25 in. on both sides of the feeder) from the animals. This ensured that the animals would not have access to the food when the device was activated at the programmed time. A touch screen keypad was connected to the automated food rack to allow for easy programming of the date and time. Once properly programmed, a start button was pressed and

the machine would function much like an automated light timer and lift the food-filled rack away from the cage feeder at the appointed time. Although there were some design and operational challenges to overcome with the initial prototype, through collaboration between the engineering and user groups, a successful automated fasting system was ultimately created.

### **PS30 Standardizing the Definition of a "Dirty" Soiled Rodent Cage**

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Customer service and cleanliness are 2 important keys to running a successful lab animal facility. After sending out a general customer dissatisfaction survey to animal users at our institution, the most common complaint received involved dirty rodent cages. A formal standardized definition of "dirty" had never been established, leaving animal care staff and researchers to determine when an individual cage needed changing. According to the *Guide for the Care and Use of Laboratory Animals*, "Solid-bottom caging usually requires sanitation at least once a week." To comply with that guideline, it is our standard schedule to change static microisolation cages once per week, with a "spot check" 2 to 4 d later, and ventilated microisolation cages every 14 to 15 d. Cages are also checked daily for flooding and other adverse conditions that may be harmful to the animal. In responding to customers' complaints, a formal definition of a "dirty" cage was needed. We photographed the bottom, open top, and side of 6 microisolation cages (3 PIV, 3 static), each containing 2 mice, 5 mice, or a dam with a litter, on consecutive days between normally scheduled changes to display the appearance and soiling of the bedding in each cage condition. Temperature, humidity, and ammonia levels were measured with each cage condition and found to be within allowed limits per the *Guide*. We tracked how many cages actually met the new standardized definition during the traditional 7 or 14 d schedule. We also looked at how number of animals/cage and age of animals (including litters) impacted the number of days before the new standard was reached. These photographs and survey results helped to define customer expectations. They also may be used to replace our current engineering-standard cage-changing schedule, with a performance based approach that meets a collective and reasonable definition of a dirty cage while improving animal welfare and reducing costs.

### **PS31 Dry Heat Sterilization of Microisolation Cages**

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Microisolation cages have been a preferred method of housing research mouse colonies since the 1980s. Large-capacity, high-vacuum steam autoclaves are used by most facilities to sterilize rodent cages. While effective, steam sterilizers are expensive to install, operate, and maintain, and they also consume significant amounts of energy and water. Installation may be difficult, expensive, or impossible when renovating an existing facility. Sterilization may be the single most costly component of the total lifecycle cost of using microisolation cage systems. Dry heat sterilization is a cost-effective alternative to steam autoclaving for sterilization of rodent cages. A dry heat sterilizer is less expensive and simpler to install and operate than a comparably sized steam autoclave, and it offers several advantages for installation in an existing facility. Dry heat sterilizers will not totally replace the need for a steam autoclave in the animal facility, but will allow for employment of smaller autoclaves that can be used less often. Dry heat sterilization is an established technology, but only recently have commercial sterilizers designed for use in an animal facility been available. This presentation will discuss experience with a dry heat sterilizer installed in an existing facility, including design, renovation, installation, start-up, validation, energy and labor costs, throughput, and advantages and disadvantages. Planners and managers looking to operate energy-efficient animal facilities should consider dry heat sterilization for both new construction and renovation projects.

### **PS32 Monitoring of Humidity, Internal, and Bedding Temperatures in Static Mouse Caging Following Steam Sterilization**

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To assess the microenvironment within static mouse cages following sterilization, rodent microisolation cages, preloaded with food and bedding, were autoclaved prior to use for housing. After sterilization, the outsides of cages were comfortable to touch within 45 min; however, we were unable to determine if the internal environment was retaining excessive heat potentially hazardous to animals placed into these cages. Polycarbonate cages were stacked (10 rows × 7 columns) in duplicate (front and back;  $n = 140$  cages) on a 3-sided wheeled-truck and autoclaved at  $\geq 121$  °F. Cages ( $n = 6$ ) were assessed to represent top, middle, and bottom rows and edges of stacked columns. Internal cage environment was measured every 10 min for 150 min after sterilization. Readings were taken using portable hygrometers that measured internal temperature (IT), bedding temperature (BT), and cage humidity (CH). At time zero, there were no differences in averaged temperatures or humidity readings between cage locations; IT = 95.9 °F, BT = 109.8 °F, and CH = 84.1%. Over time, significant differences emerged between the 6 positions. IT and BT for cages in the center row cooled more slowly than those in the bottom row ( $P < 0.05$ ). The CH in top row cages decreased more quickly compared to other cages. After 150 min, the average measures were IT = 75.8 °F, BT = 77.9 °F, and CH = 82.4%. When compared to cages that were allowed to cool overnight (IT = 72.4 °F, BT = 71.0 °F, CH = 49%) and cages housing mice (IT = 72.2 °F, BT = 70.7 °F, CH = 82%) it was concluded that 45 min was insufficient for cooling to reach acceptable temperatures after sterilization. Overall, cooling time of the cage and bedding was significantly different and dependent upon cage placement on trucks for sterilization. Our study demonstrated cages should be cooled for at least 3 h after sterilization prior to housing mice to allow for stabilization of the cage environment.

#### PS34 Tail Tattoo Identification of Specific Pathogen-Free Weanling Replacement Breeder Rats

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Proper identification of animals is necessary in rodent breeding colonies in order to ensure that the desired breeding program is followed. Identification of rodents can be achieved using several practices. Tattooing offers several advantages such as being permanent, minimally invasive, and few ID losses. However, tattooing requires specialized equipment, and can be a source of cross-contamination between animals. These problems increase in rodent colonies maintained under SPF barrier conditions, since movement of personnel, equipment, and supplies must be controlled. As a result, specialized tattoo equipment used in barrier areas must either be dedicated or sterilized prior to use. The objective of this work was to test a modified tattoo technique applied to the ventral cranial portion of the tail in weanling rats. It was developed from a technique of tattooing the paws of newborn rat pups. The procedure was approved by the IACUC, and was used on 21 to 25-d-old homozygous (nu/nu) nude male ( $n = 48$ ) and heterozygous (nu/+) female ( $n = 48$ ) rats, assigned into 4 breeding groups. Within each breeding group, males and females were assigned a number from 1 to 10. The tattoo consisted of a dot (d) of approximately 1 to 2 mm in diameter performed by injecting tattoo ink intradermally using a sterile 20G needle. The IDs were expressed using a combination of number of dots and position of the dots on the tail. A ventral portion of the tail (1 cm) distal to the hair line is divided into 4 quadrants. Distal left (DL), proximal left (PL), distal right (DR), and proximal right (PR). Number scheme is as follows: 1 = 1dDL, 2 = 1dDR, 3 = 1dPL, 4 = (1dDL + 1dPL), 5 = (1dDR + 1dPL), 6 = 1dPR, 7 = (1dDL + 1dPR), 8 = (1dDR + 1dPR), 9 = (1dPL + 1dPR), and 10 = (1dDL + 1dPL + 1dPR). Results showed that tattoos were easy to read by elevating the tail, legible for at least 6 mo, and without complications such as secondary infections. Disadvantages include the limited availability of numbers for ID. In conclusion, this tail tattoo technique can be useful in SPF barrier rodent colonies where small numbers of animal are needed as replacement breeders with the advantage that no specialized equipment is required.

#### PS35 Installation of a Distributed Decontamination System Integrated into a New BSL-3 Animal Research Facility

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This case study describes the process to design and install a gaseous chlorine dioxide decontamination system to proactively decontaminate a new BSL-3

animal research building. The facility was a new research area where the university desired to ensure that it was free of any pathogens before the research animals were brought into the facility. The university wanted to use a single decontamination system for the BSL-3 areas, as well as other animal areas. This would reduce the requirement of having to purchase more than 1 decontamination system. Therefore, a distributed installation configuration was chosen where the decontamination system could remotely decontaminate any room of the BSL-3 area without needing to be brought into that area. The decontamination system would be placed in a central location and the gas sent up to 500 ft into any particular room. To facilitate having the highest safety standards, a decontamination system criterion was established stating that it needed to provide for a complete and thorough decontamination with equipment decontaminated in the room so as not to bring contaminants into other areas. Chlorine dioxide gas was chosen because of the nature of a BSL-3 facility, requiring a thorough decontamination and penetrative ability to reach all surfaces including floor drains and HVAC vent grills. This study demonstrates the utility of using gaseous chlorine dioxide for the remote decontamination of large BSL-3 facilities with many equipment surfaces, a complex geometry of numerous rooms, and miscellaneous electronic equipment without the need to bring the generator in and out of the controlled areas.

#### PS36 Treatment of a *Giardia* Infection in a Cat Colony

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*Giardia* is an enteric protozoan found in many species with often a high prevalence in animals in research/breeding facilities. *Giardia* cysts are immediately infective and with the close proximity and number of animals housed in facilities reinfection can readily occur. Treating infections is preferred to reduce both the potential zoonotic risk and the negative impact it may have on a research study. A colony of cats tested positive when screened with a fecal *Giardia*-ELISA test. The 38 domestic short haired cats, ranging in age from newborn to 6 y of age, were housed both in cages and group housed on the floor in 4 rooms. There was regular movement, and some cats were removed during the course of testing and treatment. All cats were orally treated on day 0 with fenbendazole, 50 mg/kg daily for 7 d, mixed in a small amount of canned cat food. Both the powder and the liquid formulations of fenbendazole were used as cats had a preference. On day 0, all cats were also removed from their room, received a complete bath using a grooming shampoo and all caging, bowls, and environmental enrichment items were put through a sterilizing cage washer; the room was completely cleaned and sanitized using a quaternary ammonium disinfectant. Carpets were removed and replaced with towels and fleeces that could be laundered, and litterboxes with corncob bedding were changed daily. On day 6, all day 0 procedures were repeated. Cats were retested using the fecal *Giardia*-ELISA on days 13 to 14, 22 to 23, 44 to 45, and 126 to 127 and consistently tested negative. In comparison to 2 unsuccessful earlier attempts to eliminate the infection, disinfection of the environment, bathing of all animals, and accurate dosing of fenbendazole were critical in successfully treating the *Giardia* infection.

#### PS37 Bringing New and Developing Known Skills to the Training Staff

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Our institution has a training core consisting of 5 staff trainers and a veterinary director. Trainers are assigned different areas of specialization including husbandry, clinical, and research staff training. In addition, trainers are expected to gain a working knowledge in multiple areas so that the team members may provide support to one another. In an effort to further develop the team we instituted monthly "train the trainer" wet labs. This wet lab provides consistent expectations for current techniques and introduces new techniques or skills. Trainers suggest techniques that need to be worked on or developed and these are prioritized for practice during wet labs. Instructors who specialize in the new techniques are asked to join the wet lab as needed. We have reviewed the following techniques in our wet labs: tail nick blood draw, tail prick blood draw, and ear tagging. We have introduced the following new techniques in our wet labs: jugular blood draw in rats, sublingual blood draws in rats, rat restraint, rodent anesthesia, and penile injections in mice. Each trainer has increased and/or fine-tuned one or more technical skills. In order to measure our success each trainer evaluates



each wet lab by filling out a survey. The survey allows trainers to evaluate multiple aspects of the wet labs including impressions of the instructor, facility, comfort with new skills, and suggestions for improvement. A total of 5 wet labs have been held and all have received acceptable to excellent reviews from participants. We feel that regular meetings to practice and gain new skills are helping to develop a very well rounded training staff.

#### **PS38 A Rational Approach to Enriched Nutrition for Nonhuman Primates**

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Obesity is a concern with nonhuman primates (NHPs) housed in captivity for extended periods of time, mostly due to overfeeding and lack of exercise. This is often compounded by environmental enrichment programs that include different types of food. That is because such food is usually high in calories and added to the diet without a concomitant reduction in the number of standard chow biscuits provided. These additional foods may not meet an animal's daily nutritional needs and could result in nutrient deficiencies if not balanced with compensatory foodstuffs. Consequences of all of the above include not only obesity and related complications, such as type-2 diabetes, but also unintentional effects on the animal model that may adversely affect research data. To avoid these problems, we developed an algorithm and spreadsheet to easily determine the caloric needs of individual NHPs based on calculated basal metabolic rate and activity level. Using input variables such as body weight, activity, and dietary nutrient values, one can calculate the resultant current caloric requirements and the number of biscuits required to meet daily nutritional needs. If other foods are involved, a balanced feed ration model was incorporated to account for fruits, nuts, and miscellaneous treats, in order to better match the animal's nutritional needs and intake with the nutrient content of the various foods offered. The anticipated advantages of this approach include NHPs in better body condition and health and lower feed and labor costs by avoiding wastage.

#### **PS39 Working Safely with Animals in a BSL-4 Facility**

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BSL-4 Inserm Jean Merieux, INSERM, Lyon, France

The last 60 y have seen the emergence of many pathogenic viruses that need to be handled in high biosafety level laboratories (BSL-4) due to their high lethal rate and lack of effective treatments and vaccines. In BSL-4 laboratories, the work is performed in class II biosafety cabinets, staff must wear positive pressure suits and there is a prohibition of breakable or sharps materials. Strict application of biosafety procedures limits the biologic hazard risks for in vitro manipulations of highly pathogenic viruses. In spite of these precautions the risks remain very high when working with animals infected with BSL-4 agents. This is due to the animals themselves since they can bite and scratch, and also because of the need to work with contaminated sharps materials. To minimize the risks related with infected animals, it is important to manipulate only anesthetized animals and to apply very rigorous procedures concerning all possibly contaminated sharps materials. In our BSL-4 facility, access to experiments with infected animals is limited to a small number of workers after a long training process. All experiments are worked in pairs with very rigorous procedures. All manipulations are performed on anesthetized animals using gaseous anesthesia, except for monkeys for whom contention systems are used before parenteral injection of anesthetics. Small rodents are taken from their cages using forceps and placed in an anesthetic chamber. Ferrets are a good model for pathogenicity, treatment, and vaccinations studies concerning the flu, particularly avian flu, and SARS; however, they are among the most dangerous laboratory animals to work with due to their flexibility and ability to bite. The availability of special cages with HEPA filters and a contention system permitting the transfer of the ferrets in an anesthetic chamber without the need to touch them allows us to work safely with this animal model.

#### **PS40 Inappetence in a Chronically Catheterized Rhesus Macaque (*Macaca mulatta*)**

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A 19-y-old, 9.9 kg male rhesus macaque (*Macaca mulatta*) with a history of chronic indwelling intravenous catheterization for pharmacological substance administration was presented for inappetence of 2 d duration. Physical exam revealed recent 1.1 kg weight loss and poor body condition (BCS 2/5). Complete blood count (CBC) and serum biochemistry indicated mildly elevated ALP but were otherwise unremarkable. Aerobic and anaerobic blood cultures were negative. Rectal culture was negative for *Salmonella*, *Shigella*, and *Yersinia*. Despite nutritional support, including a high protein supplement and an increase in the amount of fresh fruits and vegetables supplied, no clinical improvement was observed. One week after initial presentation, the animal exhibited weakness upon standing. Repeat physical exam including a detailed orthopedic evaluation revealed further weight loss (0.4 kg), decreased body condition (BCS 1.5/5), moderate left coxofemoral crepitus, and mild left tibiofemoral crepitus. Pelvic radiographs revealed severe degenerative changes in the left coxofemoral joint. Survey radiographs of the thorax, abdomen, and hindlimbs were within normal limits. Medical therapy for degenerative joint disease was initiated, including carprofen (4 mg/kg PO q 24 h) and cimetidine (100 mg PO q 8 to 12 h). The patient's inappetence improved within days of initiating medical therapy and recurred when attempts were made to decrease the carprofen dose. Daily glucosamine (500 mg PO) chondroitin sulfate (400 mg PO) was added to the treatment regimen for further joint support, but effects were not sufficient to allow withdrawal of carprofen. Inappetence resolved and effective pain management was achieved using the combination of carprofen, cimetidine, and glucosamine chondroitin sulfate. Based on clinical signs and serum chemistry evaluations, specifically liver parameters, no adverse effects were associated with this regimen. Glucosamine chondroitin sulfate is commonly used in companion animal medicine for effective early management of degenerative joint disease and warrants further consideration for use in aging primates.

#### **PS41 Abdominal Mass and Pulmonary Mineralization in a Chronically Catheterized Adult Female *Macaca mulatta***

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A 16-y-old, female rhesus macaque was reported for lethargy and inappetence while on a multiyear drug evaluation study. An indwelling jugular catheter had been continuously used for 5 y. Historically, a presumptive diagnosis of endometriosis had been made based upon clinical signs and a palpable abdominal mass. Upon initial examination, the macaque was pyrexia with a palpable abdominal mass. Serum biochemistry abnormalities included hypoalbuminemia (2.5 g/dL) and elevated amylase (1,145 U/L). Radiographs revealed focal perihilar thoracic mineralization. Differential diagnoses included endometriosis, pancreatitis, gastrointestinal foreign body, neoplasia/abscess, and/or septicemia. Broad spectrum antibiotics (cefazolin and enrofloxacin), an analgesic (carprofen), an H2-blocker (famotidine), intravenous fluids, and pedialyte were provided. Blood cultures grew *Staphylococcus aureus*. The indwelling catheter was removed and a laparotomy was performed. Two uterine masses, later identified as leiomyomas, were removed by ovariectomy. No evidence of endometriosis was noted. Following surgery and antibiotic therapy, the macaque became clinically normal and negative on blood culture. Thoracic radiographs 4 wk later detected no change in the mineralization. Differentials for the persistent mineralization included pneumonia, neoplasia, dystrophic mineralization, or tuberculosis. Blood culture, 2 intradermal tuberculin tests, and a gamma interferon primate TB test were all negative. The macaque continued to be clinically normal and another experimental jugular catheter was placed. Four days after catheter placement she became lethargic, inappetent, febrile, anemic (19.9%), and bacteremic (*S. aureus*). Euthanasia was elected. The mitral valve had severe, chronic valvular vegetative endocarditis with evidence of embolic showering to distant organs. The pulmonary mineralization was associated with an occlusive thrombus with osseous metaplasia of the superior vena cava. This case demonstrates that clinical disease is often multifactorial and clinical response may not correlate with disease resolution.

#### **PS42 Isolation of *Helicobacter macacae* from a Macaque with Intestinal Adenocarcinoma**

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Motivated by the isolation of a novel *Helicobacter* from a colony of macaques in which diarrhea from chronic idiopathic colitis was enzootic, a survey of *Helicobacter* carriage was performed in a second colony without a history of diarrhea. Fifty-seven percent (20 of 35) of this latter colony were fecal-culture positive for 1 of the organisms isolated earlier, an organism subsequently named *Helicobacter macacae*. Ten years after the survey, 1 of the animals from which *H. macacae* had been isolated, a 23-y-old, intact male rhesus monkey (*Macaca mulatta*) used in neuroscience research, presented with partial inappetence and progressive weight loss. Subsequent evaluation demonstrated anemia, hypoproteinemia, hypoalbuminemia, and a palpable abdominal mass. Contrast radiography suggested partial intestinal obstruction. The animal was euthanized and a diagnosis of intestinal adenocarcinoma of the ileocecolic junction with metastasis to regional lymph nodes and liver was made. Cecal culture yielded a *Helicobacter* organism identified by 16S rRNA sequencing to be the same organism isolated earlier. Liver, small intestine, and colon were also positive by PCR for *Helicobacter*. Intestinal adenocarcinoma is the most common malignancy of aged macaques. The apparent persistence of *H. macacae* in this animal, the isolation of the bacteria from animals with colitis, and the recognition of the importance of inflammation in carcinogenesis raise the possibility of an etiologic role in the genesis of intestinal adenocarcinoma.

#### PS43 Clinical Manifestations of Amoebic Encephalitis in a Chimpanzee (*Pan troglodytes*)

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A 24-y-old female chimpanzee group housed with males and females presented with bilateral blepharospasm, cervical stiffness with mild ataxia. A complete physical examination included an abdominal ultrasound and spinal radiographs. A complete blood count (CBC) that included an erythrocyte sedimentation rate (ESR), serum chemistry panel, and urinalysis were also performed. Physical exam findings included bilateral ruptured tympanic membranes, a laceration to the sex, swelling, and pain upon cervical manipulation. The CBC demonstrated a mature neutrophilia ( $13.2 \times 10^3/\mu\text{L}$ ) and a slightly elevated ESR (33 mm/h). Serum chemistry revealed mild hypertriglyceridemia, hypercholesterolemia, and hypokalemia. Radiographs and ultrasound showed no obvious lesions. Initial differentials included trauma, meningitis/encephalitis, and neoplasia. Therapy included dexamethasone, penicillin, and ibuprofen. The chimpanzee improved clinically over the following 2 d. On day 3, she presented with head shaking and recurrence of ataxia. A repeat CBC and serum chemistry revealed a marked neutrophilia ( $31.24 \times 10^3/\mu\text{L}$ ), a mild prerenal azotemia, and a reduction of the triglycerides and ESR (22 mm/h). Antibiotic therapy was changed to include ceftriaxone and imipenem. Cerebrospinal fluid (CSF) obtained from a lumbar puncture showed a marked lymphocytic pleocytosis that was considered to be most consistent with a viral infection or the acute stage of pneumococcal meningitis. CSF titers for Herpes Simplex-1, West Nile Virus, and Arboviruses were negative. Antibiotic therapy and supportive care were continued, but the animal succumbed 3 d later. Necropsy and histopathology diagnosed a pyogranulomatous and necrotizing meningoencephalitis with large numbers of protozoal organisms consistent with amoeba. Amoebic encephalitis is an extremely rare disease in humans in the United States and most often fatal. This report demonstrates the complex clinical picture in the diagnosis of amoebic encephalitis.

#### PS44 A Genetic Basis and Varied Phenotype of "Wasting" Syndrome in a Colony of Common Marmosets (*Callithrix jacchus*)

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A 4-mo-old, female common marmoset (*Callithrix jacchus*) presented alopecia and failure to gain weight, developing lameness 2 mo later. Survey radiographs revealed osteopenia and pathologic fracture of the left femur. A custom splint was applied, but the laboratory elected euthanasia followed

by necropsy due to poor prognosis and fibrous osteodystrophy revealed in histology. This case led us to examine the possible link between bony lesions and marmoset "wasting" syndrome (MWS) in a breeding colony maintained at our institution. The classic clinical picture of MWS consists of intractable diarrhea with associated weight loss and other sequelae. Others have reported concomitant metabolic bone disease, which has been attributed to a combination of an inability to absorb sufficient dietary vitamin D through the inflamed gut and/or competitive binding of vitamin D receptors by a heat shock protein. Through radiography and archival histology, we identified bone disease in several other animals originally presenting with MWS. In addition, we identified a second disease presentation not consistent with classic MWS, but typified by bony lesions occurring in juvenile marmosets with or without diarrhea and a failure to grow. This syndrome radiographically and histologically resembles the human disease rickets. Pedigree analysis of marmosets presenting with either MWS or rickets-like syndrome suggests that not only are these 2 diseases inherited, but they may be genetically linked and could represent 2 phenotypes of the same underlying process. In addition to presenting vitamin D analyses, we will present our finding that animals with a severe form of the bone disease have high circulating levels of serotonin, the neurotransmitter through which a pathway linking GI tract and bone has recently been discovered in humans. This suggests that vitamin D may not fully explain the link between gut and bone disease in marmosets.

#### PS45 *Tyrophagus putrescentiae* Infestation in a Murine Research Facility

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The mold mite (*Tyrophagus putrescentiae*) is the most economically important and abundant mite inhabiting stored food and food-products. While evaluating mice for pruritic dermatitis, 0.3 to 0.5 mm long translucent acarids with numerous setae were identified on the rodent chow in the cage. Mites were also found on cage surfaces, in the bedding and feces, and on the mice in the cage. *Tyrophagus putrescentiae* was also identified in the sentinel cages associated with the affected mice and subsequently identified in static microisolation cages in 4 of 5 animal facilities. Mites were not found in mouse cages within barrier housing areas or in a facility using microisolation ventilated cages. Dermatitis was not a consistent finding in mice housed in cages affected by mites. Environmental sampling determined that the original source of the mites was our centralized feed storage room, which serves as a source for other feed storage rooms and facilities. The primary storage room was repainted and resealed. No antiparasitic treatment was instituted for mice in affected cages. Husbandry practices for mice were updated to include weekly changeover of feed for 4 consecutive weeks followed by complete changeover of cage feed once monthly. Nine months after the change in husbandry practices further outbreaks of mites have not been identified, nor have mites been observed during sentinel examinations. Whether *Tyrophagus* mites contribute to murine dermatitis remains unknown. The husbandry changes implemented appear to be effective at controlling *Tyrophagus* mites at the cage level. Environmental control can be complicated by the mites' unresponsiveness to certain insecticides and concerns regarding the safety of using insecticides near animal feed. To the authors' knowledge, this is the first report of *Tyrophagus* spp. infestation in a murine research facility.

#### PS46 Nail Trims versus the Previous Standard of Care for Treatment of Mice with Ulcerative Dermatitis

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From June 2007 to May 2008, there were nearly 3,000 documented cases of sickness or injury to mice used for research at our facility. Approximately one-third of those injuries involved ulcerative dermatitis, which was exacerbated by self-trauma (scratching). The standard of care prior to November 2007 did not help decrease the number of euthanasia performed resulting from these advanced lesions. This approach included systemic treatments, which affected breeding, the overall health of the animals, and in some cases, the outcome of various experiments performed on those animals. Therefore, the veterinary technicians at our



facility began to routinely perform nail trims on mice with lesions due to self-trauma. We compared the documented cases in the 6 mo prior to November 2007 (when nail trims were added) to the documented cases for 6 mo after November 2007. By comparing the cases during these time frames, we sought to evaluate whether trimming the nails increased the chances of healing and/or decreased the damage the mouse inflicted on itself, thereby decreasing the healing time. Data analysis confirmed our hypothesis that trimming the nails both increased the number of mice that were healed and decreased the time it took for them to heal. Since trimming the nails prevented the progress of the disease and eliminated the need for confounding variables such as steroids, tranquilizers, systemic antibiotics, and ultimately euthanasia, our approach will have far reaching effects industrywide on the treatment of ulcerative dermatitis. Since trimming the nails of a mouse saves time and does not adversely affect the outcome of the vast majority of experiments performed on mice, this is an ideal approach.

#### **PS47 Implementation of Self-Administered Oral Analgesic Treatment in Rats Subjected to Invasive Procedures**

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We developed an effective system of self-administered pain relief treatment in rats subjected to invasive surgical procedures. Using computer controlled automated blood sampling equipment and quantitative ELISA-measures of serum corticosterone and total corticosterone excretion, we scrutinized the stressful effect of experimental surgery on corticosterone concentrations in the circulation, urine, and feces of rats. Commercially available buprenorphine formulations for sublingual, as well as subcutaneous and intramuscular administration, were tested for efficacy in terms of their pharmacokinetics and ability to maintain unaltered corticosterone levels. Buprenorphine administered twice daily in a commercially available popular sticky nut paste for human consumption is enthusiastically consumed by rats, and resulted in long-lasting high buprenorphine concentrations in the circulation and effectively prevented procedure-mediated increases in corticosterone levels. Consequently, we are introducing self-administered pain relief as default in our units anytime rat models are used in invasive surgical procedures.

#### **PS48 Assessment of the Efficacy and Duration of Buprenorphine and Carprofen Analgesia in a Mouse Model of Acute Incisional Pain**

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Surgical incisions induce nervous system changes characterized by hyperalgesia to mechanical and thermal stimuli. The complex nature of incisional hyperalgesia necessitates the use of pertinent models to evaluate treatments for acute postoperative incisional pain. Since existing recommendations for pain management in mice are derived from nonincisional tests, we determined the efficacy and duration of 2 commonly used analgesics, buprenorphine and carprofen, in a mouse model of incisional pain. Sixty-one naïve male C57BL/6N mice were used; an incision was created on the plantar surface of the animal's right hind paw. Mice were tested for sensitivity to mechanical and thermal stimuli by sequentially applying calibrated von Frey filaments and a radiant heat source to an area adjacent to the incision. Each animal served as its own control and was tested before and after injury. Following testing, each animal received a single dose of either buprenorphine (0.05, 0.5, 1.0, or 2.0 mg/kg) or carprofen (5, 10, 20, or 30 mg/kg) subcutaneously and testing was repeated at 0.5, 1 (carprofen only), 2, 4, 6, and 8 h after drug administration. A single dose of buprenorphine at 0.5, 1.0, and 2.0 mg/kg produced analgesic onset within 0.5 h after administration, an effect that persisted in a dose-dependent manner for 2, 4, and 6 h, respectively. At 8 h, none of the doses were able to reverse hyperalgesia. Surprisingly, the commonly used buprenorphine dose of 0.05 mg/kg had no analgesic effect. Based on these findings, buprenorphine doses of 0.5 to 2.0 mg/kg are recommended for the management of acute incisional pain. There was a significant difference in the onset of analgesia after carprofen administration, ranging from 0.5 to 1 h at higher (20 or 30 mg/kg) versus lower (5 and 10 mg/kg)

doses, respectively. Thereafter, carprofen provided equipotent analgesia at all doses tested, with duration of 6 to 8 h. To avoid potential side effects of NSAIDs, carprofen should be given at 5 mg/kg q 6 h for managing acute incisional pain in the mouse. Results from these studies should be predictive of clinical efficacy and duration of these agents in mice, and used to manage acute incisional pain in this species.

#### **PS49 Sublingual Blood Collection in Unanesthetized Rats**

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When researching sublingual blood collection in rats as a possible alternative blood collection method, the technique required the animal to be anesthetized. Two people were needed for this collection — one to hold the animal, the other to hold the tongue out and bleed. Upon refining this technique, it was found that this collection can be done without anesthesia and by 1 person without assistance. Utilizing the basic gavage hold in such a way that the sublingual vein is exposed on the left or right and the animal is restrained comfortably, the mouth is rinsed with tap water to remove any contaminants, such as food particles and excess saliva. The tongue is then dabbed dry with gauze. Using a 23G or 25G needle, based on the amount of blood to be collected, the sublingual vein is punctured and blood will immediately and abundantly flow from the vein. After the appropriate amount of blood is collected, the tongue is then held off with gauze, or a cotton tipped swab. Since the tongue heals rapidly there is minimal damage to the vein, allowing it to be used for several collections in a short period of time. Also, because the sublingual vein is superficial, the hold and the initial puncture cause minimal discomfort to the animal. Additionally, initial clinical pathology testing reveals that most hematology and chemistry blood parameters (with some exceptions, such as amylase) are comparable to other more popular blood collection techniques. In conclusion, this technique can be used with minimal resources while at the same time exposing the animal to minimal damage and discomfort or risk of mortality, making it a favorable alternative blood collection technique.

#### **PS50 Wheel Running and Cage Climbing as Distinct Measures of Activity in Two Strains of Mice**

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Recent research into the effects of exercise in behavioral paradigms has shown the positive effects of free access to running wheels. Climbing on the cage top has also been shown to have important behavioral effects in multiple strains of mice. In the present study, we investigate simultaneous measures of running wheel activity and cage climbing activity in 2 strains of mice. A novel sensor was developed with which to measure cage climbing activity. Two inbred (C57BL/6 and BALB/c, 5 to 7 wk old) mouse strains were housed in conventional cages and given free access to running wheels and wire cage tops. The mice were monitored 24 h/d for running activity and cage climbing activity. C57BL/6 mice ran significantly more than BALB/c mice ( $9.6 \pm 0.4$  km/d, and  $5.2 \pm 0.2$  km/d, respectively,  $P < 0.05$ ). When wheel running activity was measured on a per hour basis, BALB/c mice ran about half the distance that C57BL/6 mice ran ( $0.22 \pm 0.01$  km/h and  $0.40 \pm 0.02$  km/h, respectively). Although BALB/c mice appeared less active than C57BL/6 mice based on running wheel activity, we observed that BALB/c mice actually were very active, spending much more time climbing the cage top than C57BL/6 mice. This study underscores the importance of using multiple measures of activity. When making conclusions about the impact of exercise or activity on behavioral, physiologic, or metabolic conditions, it could prove important to consider multifaceted measures of activity, such as cage climbing and wheel running.

#### **PS51 Mathematical Modeling for Prediction of Population Structure in Breeding Colonies of Nonhuman Primates**

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Nonhuman primates (NHPs) captive breeding programs are critical to meeting the need for animals in biomedical research, which has increased

annually since 2001. Efficient management of captive NHPs colonies requires a method to predict the prospective population with regard to both absolute numbers and population structure. The ability to predict the detailed structure of populations in terms of age, sex, and life-stage distribution would improve resource management, animal housing planning, and personnel recruitment. Such a model would also help to establish and maintain a balanced age/gender population. Several models have been widely used in ecological studies of free ranging NHPs to predict future population size. The Leslie model describes the growth of populations and their projected age distribution. The Lefkovitch Matrix is another model that considers life stage other than age classes. Both of these models, however, consider only the female population, and are not able to project the population structure in specific detail. Because of these limitations neither model is suitable for prediction of NHP populations in research settings. We have modified the Leslie/Lefkovitch matrix, a combination of 2 mathematical models, to allow for projections of age class, life stage, and gender, as well as simulations of the effect on population structure of various demographic and management parameters. A programming system and a spreadsheet application were used to generate our model and project 10 y of future population in large socially housed breeding colonies of rhesus monkeys (*Macaca mulatta*) at the California National Primate Research Center. With this model we were able to graph the population trajectory for age, life stage, and gender as well as the population as a whole. Furthermore using a mathematical rather than a statistical model, allows us to apply this model to newly established colonies with a small initial population.

#### **PS52 Effect of Playful Handling during Routine Care on Anxiety-Related Responses of Laboratory Rats**

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We hypothesized that playful handling by caretakers (mimicking playful social contact between rats) reduces anxiety and minimizes adverse effects of individual housing. We assessed caretaker handling effects on male Sprague-Dawley rats (*Rattus norvegicus*; Sim:(SD)fBR Albino found free of internal/external parasites and disease via quarterly serology and parasitology and yearly necropsy evaluations) ( $n = 72$ ) exposed to 1 of 2 handling treatments: 1) Standard (control) handling, during weekly cage cleaning only; 2) Tickling 2 min daily for 3 wk starting at 25 d of age. Rats in both conditions were housed individually, in pairs, or in triplets. During week 4 of the experiment, we collected fecal samples to assess corticosterone levels, and measured the rats' anxiety-related behavior in an emergence test. All rats were then individually housed, with standard handling, for the next 3 wk and retested during week 8. Ease of handling was assessed during cage cleaning using a handling score. When handled during cage cleaning, tickled rats struggled less than control rats ( $P < 0.0001$ ). Tickling did not affect corticosterone levels, or behavior in the emergence test, in either week 4 or 8. All rats had lower corticosterone levels in week 4 than 8 ( $P < 0.0001$ ), and individually housed rats had higher corticosterone levels than pair- and triplet-housed rats ( $P = 0.02$ ). During week 4, control triplet-housed rats had shorter latencies to put their head out ( $P = 0.01$ ) and emerge from the dark box ( $P = 0.02$ ) compared to control individually housed and control pair-housed rats, respectively, in the emergence test. These effects were not significant in week 8. In conclusion, playful handling (that is, tickling) did not reduce anxiety, or have lasting benefits for group-housed rats that were subsequently housed individually, but our results suggest that it did reduce fear of familiar humans.

#### **PS53 The Use of ATP-Based Methods or RODAC Plates for Monitoring the Effectiveness of Sanitation Procedures in an Animal Facility**

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The *Guide for the Care and Use of Laboratory Animals* requires that research animal facilities have an environmental monitoring program to determine the effectiveness of sanitization procedures. The method(s) of choice is not always clear and depends on the objectives and quality of the research program as well as economic factors. The most widely used methods are the swab rinse method and the replicate organism detection and counting (RODAC) method. The adenosine triphosphate (ATP) bioluminescence

testing systems are widely accepted for monitoring hygiene levels in food production and processing environments. These ATP systems are gaining in popularity for assessing the effectiveness of sanitation procedures in animal facilities. This report compares: 1) initial investment costs of 3 automated ATP systems; 2) cost of 10 swabs for each ATP system versus 10 RODAC plates needed to monitor a sanitized animal room; and 3) advantages and disadvantages of each method. The ATP systems provide results within minutes and measure cleanliness by detecting organic matter, food residue, and the presence of microbial agents. The ATP systems are expensive and do not enumerate or identify microbes. The RODAC plate method enumerates and identifies viable microbial agents with minimal investment costs. Results from RODAC plates require 24 to 48 h. It was concluded that animal research facilities differ, and no one method fits all environmental monitoring programs. What to monitor, frequency of monitoring, and methods to be used will be determined by the budget and the goals of the research program.

#### **PS54 A Novel Ergonomic Procedure Cart**

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With the cost of lost productivity due to injury and medical treatment associated with work-related injuries continuing to rise at a rate exceeding inflation, ergonomics is an increasingly important design consideration in the manufacture and use of animal manipulation apparatus. Work-related musculoskeletal disorders account for one-third of all occupational injuries and illnesses reported by employers, constituting the largest job-related injury and illness problem in the United States. Furthermore, indirect costs, such as lost productivity, retraining, and sick or administrative time, can be at least 4 to 10 times more than the direct costs. The National Research Council found a clear relationship between musculoskeletal disorders and work, and between ergonomic interventions and a decrease in such disorders. Animal care personnel in our department were experiencing lower back pain when bending over a standard utility cart to evaluate quarantined nonhuman primates (NHPs). NHP testing consisted of blood draws and injections and was done once every other week during a 6-wk quarantine period. To establish more comfortable working heights, a standard utility cart was affixed to a hydraulically height-adjustable base and evaluated by technical staff while testing 16 NHPs over a span of 2 to 2.5 h of continuous work; no lower back discomfort was experienced. Consequently, a mobile, height-adjustable work surface was built to accommodate personnel needing different work surface heights in order to maintain proper posture and avoid lower back strain. The cart was also used for regular weekly blood draws from post quarantine NHPs, as well as thrice yearly TB tests performed on the entire population of 60 animals. It was also found that different tasks were more comfortably done at differing heights by each worker, thereby enhancing the value of a height-adjustable procedure cart.

#### **PS55 Creating a Cost-Effective Housing Solution for Large USDA-Covered Species**

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Our institution was faced with a growing swine population and a limited budget for facility renovations. A large 523 ft<sup>2</sup> irregularly shaped animal holding room was selected as the trial room. The room was composed of epoxy coated flooring and painted cinder block walls. The room was prepared by coating the floor with 0.25 in. polyurethane coating material commonly used as pickup truck bed liner. A series of 3 gates that could be configured into a main pen entrance and a small holding pen were installed in the front of the room, 6 ft inside the entrance door. These gates were made of powder-coated steel. The walls of the room were lined with powder-coated sheet metal wall panels to a height of 6 ft. The existing floor drains were covered by a removable cap to prevent material from inadvertently clogging the drain. Once completed, the room provided 365 ft<sup>2</sup> of animal holding space, sufficient for up to eighteen 100 kg swine according to the *Guide for the Care and Use of Laboratory Animals'* minimum floor space requirements. Using this room, swine can now be housed in large groups, which allows for social interaction and lets them exhibit species-specific behavior by placing

bedding material in parts of the room allowing the pigs the opportunity to forage. The room is easily cleaned by washing waste material down the existing floor drains. Total design and construction of this free range room was just under US\$20,000 compared to traditional raised flooring pens quoted at over US\$100,000 for this room and providing suitable housing space for only eight 100 kg swine. This free range concept allows for maximum utilization of total floor space, which can be applied to many other animal species. The materials were all obtained from local vendors and installed by the institutional facility maintenance personnel.

#### **PS56 Impact of Nesting Material on Mouse Thermoregulation and Variability**

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In laboratories, mice are housed at 20 to 24 °C, which is below their thermoneutral zone (26 to 34 °C). Thus mice are chronically cold stressed, which alters behavior, metabolism, body composition, and body core temperature. These physiologic changes reflect impaired wellbeing and affect scientific outcomes. We hypothesized that nesting material allows mice to alleviate cold stress by controlling their thermal microenvironment within the cage. We predicted that nesting material would insulate the mice, reduce heat loss, and reduce body core temperature (cold animals compensate for heat loss by raising body temperature). We housed naïve C57BL/6, CD-1, and BALB/c mice (24 male and 24 female per strain in groups of 3) in standard cages at 20 °C either with or without 8 g of nesting material for 4 wk. Nest sites were scored daily based on a 1 to 5 scale. Body core temperature was followed using intraperitoneal radio telemetry from 1 mouse per cage. The nest sites were assessed using a thermal imaging camera once a week, and the heat conserved by insulation calculated. During weekly cage cleanings fresh nesting treatment was provided. Analyses used GLMs with post hoc contrasts. Mice given nesting material built better nests ( $P < 0.05$ ) and their body temperature was significantly lower than controls ( $P < 0.05$ ). The time of day most affected by the nesting treatment was as the lights turned on ( $P < 0.05$ ). During this period body temperature dropped more quickly as the mice began their period of inactivity. Nest sites were significantly better insulated when mice had nesting material ( $P < 0.05$ ), with approximately 2 °C conserved on average. Insulation was dependent on strain ( $P < 0.05$ ) and location within the nest ( $P < 0.05$ ). Therefore mice appear to be in a negative thermal balance under moderately cool standard laboratory conditions. However, given nesting material, they can alleviate this thermal stress and reduce heat lost to the environment by building a nest appropriate to their specific thermal needs.

#### **PS57 Breeding Colony Policy Changes Due to Pinworm Outbreak: Quarantine with Mandatory Fenbendazole Chow Treatment**

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After a recent pinworm outbreak in our murine breeding colony, a secondary quarantine process, which included mandatory treatment with fenbendazole, was initiated (excluding approved commercial vendors). Fenbendazole is commonly used to treat pinworm (*Aspicularis* and *Syphacia*) infections in mice and rats; it is a useful agent for prophylactic treatment of subclinical pinworm infections. Fenbendazole is reported to have a wide margin of safety, yet there is limited information concerning its effects upon breeding in transgenic and knockout mice. We observed temporary decreases in litter sizes and interlitter frequencies in various transgenic and knockout mice after initiating fenbendazole treatment in quarantine. With our original policy, mice could enter the main breeding colony with negative serology and endoparasite results within the last 30 d (routine dirty bedding sentinel surveillance). All mice were placed on a 6 wk prophylactic pinworm treatment utilizing fenbendazole chow (150 ppm) alternating weekly with irradiated rodent chow. Sentinel serology for rodent specific pathogens and direct cecal examinations for endoparasites were performed. Only mice that completed fenbendazole treatment in quarantine and maintained a specific pathogen-free status were transferred into the main breeding colony. Alternatively, investigators had the option to rederive their mice via embryo transfer and not receive fenbendazole treatment, but undergo a 6 wk surveillance period in quarantine prior to acceptance into the main

breeding colony. Mice were allowed to breed while in quarantine. Due to the observed effects on litter sizes and interlitter frequencies, our future plans include developing a website to document breeding performance data for transgenic and knockout mice treated with fenbendazole chow. Since implementing our new quarantine policy with mandatory fenbendazole treatment, no further rodent pathogenic outbreaks have occurred within our breeding colony.

#### **PS58 Breeding Performances as Welfare Indicator: A Comparative Study on C57BL/6J Mice in Three Different Individually Ventilated Caging Systems**

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The increasing need to use genetically modified mice as a model for many human diseases has led to the requirement of raising the breeding colonies quickly and efficiently. We selected breeding performances, among other welfare indicators, to evaluate the impact of different housing systems and their respective microenvironments. The 3 individually ventilated caging systems in our trial are characterized by different solutions for cage air inlet and exhaust. System 1, operating at 30 air changes per hour (ACH), has no air handling unit and relies fully on a negative flow of air through the connection to the room air exhaust. System 2, operating at 50 ACH, has 2 air handling units mounted on top of the rack and the air is injected into the cage at animal level. System 3, operating at 75 ACH, has an air handling unit connected to the rack by flexible silicone hoses and the air enters and exits the cages from the rear of the plastic top. C57BL/6J mice, were selected for the study; thirty breeding trios, 8-wk-old, were distributed into the IVC systems and monitored for their breeding performances over a period of 6 mo. The following parameters were considered: litter size at birth, litter size at weaning, body weight of pups at weaning and at 5 wk of age, intracage ammonia, oxygen, carbon dioxide, temperature and relative humidity, and bedding weight at cage change. Analysis of breeding performances showed a 10% higher production efficiency index in the 2 systems at 75 and 30 ACH versus that at 50 ACH. The ammonia level was 3 to 4 times higher in the system at 30 ACH versus the other 2 starting from day 9, and the mean weight of dirty bedding recovered during the 6-mo trial was from 6% to 10% higher in systems operated respectively at 50 and 30 ACH, showing an inverse correlation between ACH and moisture content in the substrate. Our findings suggest that different technical solutions for cage ventilation and performances can lead to consistent differences in the relative microenvironments and some interferences with breeding performances.

#### **PS59 Development of Glucose Testing Protocols to Identify Prediabetic Nonobese Diabetic Mice for Interventional Treatment**

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Research in the diabetes field suggests that the best time to begin treatment to prevent or reverse diabetes may be during the prediabetic stage. Glucose tolerance tests (GTT) can be used to identify animals likely to convert to hyperglycemia; however, there is a lack of consensus as to the best age to initiate testing and definition of glucose intolerance. To standardize protocols for testing of agents, we focused on the nonobese diabetic (NOD) mouse model. Groups of mice at either 12, 14, or 16 wk of age were injected intraperitoneally with 2 g/kg glucose. Blood glucose levels were measured at 0, 30, 60, 90, or 120 min relative to glucose injection. The areas under the curve (AUC) were calculated for each animal for the whole 120 min testing period and for each 30 min block of time. Animals were then held for 30 d after glucose tolerance testing to determine which animals became diabetic. The sensitivity, specificity, and positive predictive value for each AUC measurement were calculated. At 12 wk of age, 7 of the 50 animals became diabetic within 30 d after GTT. However, it was not possible to predict which animals would become diabetic since the AUC measurements from 12-wk-old animals that eventually became



diabetic or remained nondiabetic were equivalent. At 14 wk of age, 13 of the 50 mice became diabetic; 9 of these were predicted to become diabetic while 4 mice that were not predicted to become diabetic developed hyperglycemia (positive predictive value = 0.4737). At 16 wk of age, 13 of the 50 mice became diabetic; 8 were predicted, while 5 became diabetic that were not predicted (positive predictive value = 0.5000). When 0 to 30 min and 30 to 60 min AUC calculations were used to predict diabetes onset for the animals that underwent GTT at 14 wk of age, the 0 to 30 min AUC had a positive predictive value of 0.4783 and the 30 to 60 min AUC had a positive predictive value of 0.3913. We observed that using the AUC calculated for 0 to 30 min in 14-wk-old mice that underwent GTT exhibited the highest sensitivity in predicting the subsequent development of diabetes. Use of this protocol to select animals for testing interventional treatments will aid in the testing of new agents.

#### **PS60 Development of Protocols to Optimize Treatment of Spontaneous Type 1 Diabetes in Nonobese Diabetic Mice**

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Preclinical studies for testing of agents to treat or prevent type 1 diabetes (T1D) have relied on the nonobese diabetic (NOD) mouse. Standardized protocols for maintaining near normal glycemic levels in diabetic rodent models are lacking. Therefore, we developed a standardized protocol (SP) for maintaining glycemic levels in diabetic mice. Factors considered in the development of this SP included: type of insulin, dose, time of dose, frequency, and percentage of daily dose relative to fed state (lights off). We first evaluated several insulins, protamine zinc insulin, Humulin 50/50, or Humulin 70/30 by injecting groups of 6 to 15 mice subcutaneously, QD, BID, or TID to determine a total daily dose requirement of insulin. Glucose levels were determined at various times post insulin injection. These studies established that insulin would have to be injected frequently to maintain glycemic control in NOD mice. These results also showed that mice with duration of diabetes of less than 10 d responded better to insulin treatment. We next evaluated the effect of continuous insulin delivery on blood sugar levels in acutely (< 10 d) diabetic mice. Groups of 6 to 15 mice were implanted subcutaneously with 14 d osmotic pumps filled with Humulin R insulin, releasing either 0.2, 0.3, or 0.4 U insulin/24 h. Blood sugar levels were measured daily for up to 27 d post pump insertion and mice underwent intensive blood glucose monitoring periods on days 3, 7, and 14 post pump insertion. In mice implanted with pumps releasing 0.2 U/d, blood glucose levels were never stably below 200 mg/dL, although average blood glucose levels were below this level on some days. In contrast, in mice implanted with pumps releasing 0.3 and 0.4 U/d, average blood glucose levels were consistently below 200 mg/dL beginning at day 5 and day 1, respectively. Hypoglycemic events correlated with the dose of insulin used; they were more frequently observed and more severe in mice treated with 0.4 U/d insulin pumps. The 0.3 U/d dose provided the best control with the fewest hypoglycemic events. Use of this standard protocol should aid in the testing of agents to prevent or reverse diabetes.

#### **PS61 Gestational Blood Pressure Regulation and Pregnancy Outcome in the Nonobese Diabetic Mouse**

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Pregnant women with type 1 diabetes (T1D) are at high risk of developing preeclampsia, a late gestational hypertensive disorder. The pathophysiology of this disease process is still relatively unknown and there are no established mouse models of gestational hypertension linked to T1D. Using radiotelemetry, we sought to investigate blood pressure regulation in the pregnant T1D nonobese diabetic (NOD) mouse and its effect on fetal outcomes. Female NOD mice were implanted with PA-C10 radio transmitters and given 2 wk recovery. Glycemia was assessed via tail vein blood with glucose test strips every 3 d. Hyperglycemic (defined as > 15 mmol/L) and control normoglycemic, age-matched females were mated to normoglycemic NOD males. Mean arterial pressure (MAP), heart rate (HR), and animal

activity data were collected across pregnancy. Pre-pregnancy baseline measurements did not differ between groups. MAP of normoglycemic NOD mice declined from gestation day (gd) 5 to 10, and progressively increased back to baseline until parturition, a pattern we see in normal inbred mice. Hyperglycemic NOD females had a similar declining MAP to gd9 but MAP failed to increase over the second half of pregnancy. HR of normoglycemic NOD mice increased slightly in early pregnancy, started to decline at approximately gd11, and reached its minimum at gd17. In contrast, HR in hyperglycemic NOD mice declined from mating, reaching its minimum at gd17. Activity levels decreased throughout pregnancy in both groups. Offspring of hyperglycemic mice were severely growth restricted and had neural tube defects. In conclusion, rather than developing a hypertensive phenotype in pregnancy, hyperglycemic NOD mice develop a hypotensive phenotype, in part related to a deficit in cardiac adaptation. Thus, careful assessment of circulatory changes during gestation in mice provides an experimental approach to assess critical regulatory mechanisms.

#### **PS62 Establishment of a Nonobese Diabetic Mouse Colony**

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To support a preclinical immunotherapy program for type 1 diabetes (T1DM), a nonobese diabetic (NOD) mouse colony was established. Inhouse breeding is required to produce a continuous supply of mice with new onset diabetes for testing. The maintenance and management of prediabetic, frankly diabetic, and pregnant NOD mice provide special challenges. These issues include management of pregnancy, avoiding genetic drift, and control of hyperglycemia. Additionally, mice must be maintained in a barrier setting to avoid infections. These can increase IL-12 and prevent the onset of T1DM. Genetic drift is avoided by the incorporation of vendor derived female breeders each generation. Prediabetic mice are screened for the onset of T1DM by blood glucose determination using a hand held glucometer and a tail nick, weekly beginning at 10 wk of age. At least 80% of female mice develop T1DM between 12 and 20 wk of age. Normal mouse blood glucose (bg) values are less than 150 mg/dL. New onset T1DM was diagnosed based on a screening value of greater than 300 mg/dL, confirmed by 3 consecutive daily values greater than 300 mg/dL. Newly diabetic female mice are enrolled in intervention studies. With this criterion, the spontaneous reversion rate is less than 2.5%. Pregnancy in diabetic NOD mice is associated with severe fetal and maternal wastage. To avoid this, breeders are limited to 2 litters and culled at 14 wk of age. Pregnant females are tested at time of first litter wean or obvious cage wetting. If deemed diabetic, pups are fostered at time of birth and female euthanized. Mice in intervention studies have blood glucose checked twice daily and receive long acting human glargine insulin twice a day based on a sliding scale for blood glucose values greater than 250 mg/dL. Reversion is defined as blood glucose less than 250 mg/dL without insulin therapy. In this model the best immunotherapy results in reversion of greater than 80% of the mice treated.

#### **PS63 Oral Delivery of Insulin Using Poly (PEGDMA-MAA)-Based Nanoporous Microparticles**

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An oral insulin delivery system based on copolymers of polyethylene glycol dimethacrylate and methacrylic acid was developed and its activity was tested in nonobese diabetic rats. Polyethylene glycol dimethacrylate (PEGDMA) of different molecular weights were synthesized by esterification reaction of polyethylene glycol with methacrylic acid (MAA) in the presence of acid catalyst. PEG dimethacrylate of molecular weights ranging from 400 to 4,000 were copolymerized with methacrylic acid by suspension polymerization to obtain pH sensitive hydrogel microparticles. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) revealed that the diameter of poly (PEGDMA:MAA) microparticles increased with increasing molecular weight of polyethylene glycol dimethacrylate used for synthesis, AFM studies also revealed the nanoporous nature of hydrogel microparticles. Insulin was loaded into the hydrogel microparticles by partitioning from concentrated insulin solution. In vitro release of insulin from preloaded microparticles were studied by simulating the condition of gastrointestinal tract, which showed the minimal insulin leakage in acidic (pH = 2.5) and significantly

higher release in basic (pH = 7.4) environment. Gamma scintigraphic studies also demonstrated minimal release of insulin in the stomach but significant release in the intestine. This study also revealed the desired muco-adhesive property of the copolymer in the intestine. Animal studies were carried out to investigate the ability of insulin loaded hydrogel microparticles to influence the blood glucose levels in the diabetic rats. Blood glucose level reduced in animals that received the insulin loaded hydrogel microparticles and the effect lasted for 8 to 10 h. It was also observed that administration of 2 capsules per day of poly (PEGDMA4000:MAA) hydrogel microparticles containing 80 IU/kg of insulin dose was sufficient to restrict the blood glucose level in fed diabetic rats between 100 to 300 mg/dL. Toxicity and histopathologic studies revealed prospects of the formulation.

#### **PS64 Advancing Metabolic Phenotyping: Moving into the 21st Century**

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With the introduction of more complex and automated metabolic phenotyping systems, which give repeatable results using fewer animals, we are now looking at the next steps forward. We will show 2 aspects of high throughput metabolic phenotyping. In the first section we will describe how to create an environment to challenge laboratory animals' metabolism using temperature. Using this new technology we can look at energy expenditure and food consumption of genetically modified animals at temperatures ranging from 4 to 34°C. At cold temperatures we considered brown adipose tissue (BAT) activation. At higher temperatures we looked for a thermoneutral temperature so that we could see any small changes in metabolic rate as we took out the variability of energy expenditure to maintain body temperature. We will also discuss redesigning metabolic phenotyping cages to meet the demands of working at different temperatures. This new system is both user friendly and a robust design for easy cleaning, allowing for rapid changeover of animals to increase the throughput without requiring extra staff. Working with a scientific laboratory and medical equipment design company, we have now produced a system based on the animals' home cages, so there is no stress response. This presentation will be of relevance to any institution that does metabolic phenotyping. We will cover the problems that occurred and how they were overcome and show the different results we are achieving using this equipment. We will also explain why we created the temperature controlled environment and decided to redesign the metabolic phenotyping cages.

#### **PS65 Validation of a Continuous Glucose Monitoring System for Use in Dogs and Pigs**

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Monitoring changes in blood glucose concentrations in response to manipulations of the mechanisms of glucose homeostasis is essential in laboratory species used for diabetes and diabetes-related research. In dogs and pigs used for this research, blood glucose monitoring was done by obtaining multiple peripheral blood samples and measuring glucose. However, this can be problematic due to the number of blood samples necessary to obtain useful information, discomfort to the animal, and potential physiologic fluctuations of blood glucose due to animal handling. Recently, a commercially available continuous glucose monitoring system (CGMS) designed for use in humans, which avoids these complications, was introduced. The CGMS measures interstitial glucose in the skin, is minimally invasive, and capable of providing very detailed glucose information (288 readings per 24 h) over several days. The goal of this study was to validate this CGMS for use in dogs and pigs. A CGMS was placed on 6 clinically normal pigs and 4 normal dogs for a minimum of 24 h (up to 48 h) and properly calibrated. Over the course of attachment, blood glucose values were determined with a handheld glucometer and compared to paired interstitial glucose readings from the CGMS (dogs:  $n = 20$ , pigs:  $n = 31$ ). To examine the response of interstitial glucose to rapid blood glucose fluctuations, 1 pig and 1 dog were subjected to either an oral or intravenous bolus of glucose. In both species, there was excellent correlation between paired blood and interstitial glucose concentrations (pigs:  $r = 0.81$ ,  $P < 0.001$ ; dog:  $r = 0.97$ ,  $P < 0.001$ ). Also, interstitial glucose changed in response to both an oral and intravenous bolus of glucose in both species. It was concluded from this study that the

CGMS is a valid method for monitoring glucose changes in dogs and pigs, has advantages over traditional methods, and may prove to be helpful in the study of diabetes.

#### **PS66 Embryonic Pig Pancreatic Tissue for the Treatment of Diabetes: Proof of Concept in Nonhuman Primates**

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Allogenic islet cell replacement can restore normoglycemia in type 1 diabetes; however, the shortage of available organs for transplantation continues to severely limit this therapeutic option. A potential solution for this shortage is the transplantation of pig organs and tissues. However, the immunologic barriers to xenotransplantation are formidable. One approach to address the vigorous immune rejection associated with xenotransplants is the use of embryonic precursor tissue, which induces and uses host vasculature upon its growth and development. Recently, we showed in mice that embryonic pig pancreatic tissue from day 42 exhibits favorable properties as a beta-cell replacement therapy. We now demonstrate the proof of concept in 2 diabetic cynomolgus monkeys, followed for 393 and 280 d, respectively. Thus, a marked reduction of exogenous insulin requirement was noted by the fourth month after transplantation, reaching complete independence from exogenous insulin during the fifth month after transplantation, with full physiologic control of blood glucose levels. The porcine origin of insulin was documented by a radioimmuno assay specific for porcine C-peptide. Furthermore, the growing tissue was found to be predominantly vascularized with host blood vessels, thereby evading hyperacute or acute rejection, which could potentially be mediated by preexisting anti-pig antibodies. Durable graft protection was achieved and most of the late complications could be attributed to the immunosuppressive protocol. While fine tuning of immune suppression, tissue dose, and implantation techniques are still required, our results demonstrate that porcine E-42 embryonic pancreatic tissue can normalize blood glucose level in primates. Its long term proliferative capacity, its revascularization by host endothelium, and its reduced immunogenicity, strongly suggest that this approach could offer an attractive replacement therapy for diabetes.

#### **PS67 Iron Metabolism and Oxidative Stress in Insulin Resistant and Diabetic Monkeys**

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Oxidative stress is a key feature of type 2 diabetes (T2D) which may exacerbate complications such as pancreatic beta cell destruction, glomerulonephropathy, and vascular damage. Studies suggest that iron, a strong pro-oxidant, plays a key role by increasing reactive oxygen species. The goal of this study was to investigate the effect of naturally occurring increases in glucose and insulin resistance (IR) on iron metabolism and oxidative stress in nonhuman primates (NHPs). Plasma and serum were collected from 3 cohorts of NHPs, including naturally occurring diabetics ( $n = 18$ ), insulin resistant (IR,  $n = 21$ ), and nondiabetic ( $n = 19$ ) cynomolgus macaques. Components of iron metabolism, glycemic indices, markers of inflammation, and oxidative stress were determined. Serum ferritin, an indicator of total body iron stores, was observed to be significantly higher in diabetics and IR monkeys compared to nondiabetic monkeys ( $13.34 \pm 1.7$ ,  $13.48 \pm 1.3$  versus  $1.86 \pm 0.3$  ng/mL,  $P < 0.0001$ ,  $P < 0.0001$ , respectively). Transferrin, a key transport protein of iron, was significantly higher ( $237 \pm 5.6$  versus  $195 \pm 7.3$ ,  $209.3 \pm 4.3$  mg/dL,  $P < 0.001$ ,  $P < 0.001$ , respectively) in diabetics compared to IR and nondiabetic monkeys. Oxidative stress, as determined using a TBARS assay for lipid peroxidation, was significantly higher in diabetic and IR monkeys than nondiabetic ( $18.47 \pm 2.3$ ,  $10.1 \pm 0.7$  versus  $5.1 \pm 0.2$  nmol/mL,  $P < 0.0001$ ,  $P < 0.01$  respectively). Superoxide dismutase, an enzyme that inactivates free radicals, was found to be significantly lower in diabetic compared to IR and nondiabetic monkeys ( $52.22 \pm 3.6$  versus  $70.1 \pm 1.4$ ,  $72.0 \pm 1.9$  units/mL,  $P < 0.0001$ ,  $P < 0.0001$  respectively). Measurement of C-reactive protein, a marker of inflammation, resulted in significantly higher values in diabetics and IR than nondiabetic monkeys, ( $1679 \pm 196$ ,  $1430 \pm 210$  versus  $520 \pm 70$   $\mu$ g/mL,  $P < 0.0001$ ,  $P < 0.0001$ , respectively). In conclusion, both T2D and IR have increased markers of oxidation, ferritin, and inflammation compared to nondiabetics. Additionally, T2D also have less antioxidant activity, likely making them less able to manage oxidative stress.

## PS68 Use of Remote Controlled Drug Delivery Capsule, Gamma Scintigraphy, and Vascular Access Ports to Refine Preclinical Beagle Dog Studies

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In type 2 diabetes, patient's hormone secretion in the gut is affected. Glucagon-like peptide 1 (GLP-1) has decreased secretion but patients maintain normal response. Research has shown that activation of compound X by bile acids in the human colon increases GLP-1 release. As a possible therapeutic treatment for type 2 diabetes, compound X was evaluated for its effects on glucose-induced secretion of gut hormones in the dog jejunum. A nonrandomized, 4-way crossover design using conscious beagle dogs was used. Dogs were fitted with a portal vein vascular access ports (VAPs) for additional pharmacokinetic (PK) sampling due to the rapid breakdown of compound X peripherally. The jejunal dosing was achieved using a remote-controlled, radiolabeled delivery capsule, tracked using gamma scintigraphy and released by external activation. Seven blood samples were collected from each animal postdose via cephalic catheter and VAP. Resulting glucose and insulin profiles were similar to previous terminal studies, with a 46% increase in GLP-1 secretion. These refinements reduced the number of dogs to  $n = 7$  needed to obtain adequate statistical power, reduced dosing volume by 14.7 mL, reduced the variability in the data from 50 to 20 mg/dL, and delivered an exact dose of compound X to the dog jejunum. By using imaging tools in combination with pharmacokinetic analysis, the project team was able to observe the in vivo release and dispersion of compound X when dosed regionally. This study further improved the study design due to the following 4 refinements: a 4-way crossover study design (each beagle served as its own control), gamma scintigraphic imaging (reliable, noninvasive method), capsule (direct dosing to a specific region), and VAP sampling (allowing for nonterminal/crossover studies.)

## PS69 A New Nude Mouse Model for Human Hypertrophic Scar

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Hypertrophic scar (HTS) is a fibroproliferative disorder that results in excessive deposition of collagen and other extracellular matrix molecules following damage to the deep dermis by thermal injury or other forms of trauma, often causing severe cosmetic and functional impairment. Research and treatment are difficult because of the lack of ideal animal models. In this study, a new HTS model is now established that involves engrafting split or full thickness human skin onto the backs of nude mice. Animals observed developed raised, firm, and reddish scars 2 mo after transplantation. Quantification of dermal thickness using histology and micromer measurement indicates raised engrafted skin after 2, 4, and 7 mo after engraftment compared to normal split and full thickness skin. In contrast, thickening was not observed in engrafted rat skin. Histologic analysis using Masson trichrome demonstrates increased dense accumulations of thick collagen bundles in the dermis in both split and full thickness skin engraftment. Staining cells with toluidine blue and an antibody for F4/80 indicates increased murine mast cell and macrophage infiltration in engrafted skin. Quantification of fibrocytes in engrafted skin samples reveals increased murine fibrocytes compared to normal skin. Moreover, engraftment with split thickness skin had significantly more macrophages, mast cells, and fibrocytes than full thickness skin engraftment. Real time PCR analysis indicates significantly elevated mRNA levels for type 1 collagen, TGF- $\beta$ , CTGF, HSP47 in both engrafted skins. This data demonstrates that engrafted human skin onto nude mice developed a hypertrophic like scar and that an intrinsic human skin property dictates HTS formation. Interestingly, engrafted split thickness skin developed a greater percentage of scars than full thickness skin grafts. Furthermore, inflammatory cells and bone marrow fibrocyte infiltration and interaction with resident skin cells may play critical role in HTS development in this animal model.

## PS70 Correlation of Serum Activities of Liver Enzymes Alanine Aminotransferase and Aspartate Aminotransferase to Liver Mass in Normal Mice

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Serum activities of the hepatocellular leakage enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are widely used to assess damage to the hepatic parenchyma in mice. Activity for both enzymes is found primarily in the hepatocyte cytosol but can be found in muscle as well. Following hepatocellular injury or alterations in hepatocyte membrane permeability, these enzymes escape from the cytoplasm to the serum where activity can be measured. The magnitude of serum ALT and AST activity is believed to be dependent on intrinsic tissue activity, enzymes half-life, and extent of damage to the liver when injured. Occasionally, increases in ALT and AST serum activities have been observed in the absence of overt histopathologic abnormalities to the liver. In addition, anecdotal evidence has suggested a relationship between liver mass and serum enzyme activities. Based on these observations, properly interpreting changes in serum ALT and AST activities as they relate to hepatocellular damage can be challenging. We compared liver mass to serum ALT and AST activities in normal mice. Thirty-one mice of different strains were euthanized for an unrelated study. Immediately following euthanasia, the mice were weighed, a blood sample was obtained, and the liver extracted in whole and weighed. ALT and AST serum activities were determined using an automated chemistry instrument. There was a strong correlation between body weight and liver weight ( $r = 0.83$ ,  $P < 0.001$ ) and between serum ALT and AST activities ( $r = 0.89$ ,  $P < 0.001$ ). However, there was no correlation between liver mass and serum activities of ALT or AST ( $r = 0.04$ ,  $P < 0.81$ ;  $r = -0.201$ ,  $P < 0.278$ ). These findings suggest that liver mass should not be considered a confounding factor when interpreting serum hepatocellular leakage enzyme activities as it relates to liver pathology.

## PS71 Cloning and Expression of Major Antigenic Proteins for the Development of Pinworm Serology in Mice

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Affinity chromatography using IgG from pinworm-infected mice was used to isolate proteins in *Syphacia obvelata* and *Aspiculuris tetraptera* that elicit a natural murine IgG response. Peptide sequences of the proteins were determined by ion impact mass spectroscopy and these sequences were compared with the *C. elegans* genome bank. Two candidate proteins with molecular weight of approximately 65kDa were chosen for development of recombinant antigen for serologic testing for pinworms. Reverse genomics using nematode codon bias defined oligonucleotide primer pairs to amplify mRNA encoding one of the candidate proteins separately from *S. obvelata* and *A. tetraptera*. Reverse transcription PCR amplified several alternatively spliced mRNAs encoding several forms of the target protein. The largest cDNA, 1446 nucleotides encoding 482 amino acids, was sequenced and cloned into a baculovirus expression system engineering polyhistidine residues at both the C-terminal and N-terminal ends of the recombinant protein. The cloned recombinant protein was expressed in SF9 insect cells and purified using nickel-NTA coordination complex columns under strongly denaturing conditions. The recombinant proteins have an apparent molecular weight of 65kDa and bind IgG antibodies from the serum of pinworm-infected mice when analyzed by Western blot. Six  $\mu$ g of recombinant protein in 50  $\mu$ L carbonate buffer was ionically bound to microtiter plates rows A, C, E, and G for ELISA. The same protein concentration derived from supernatant of control SF9 insect cultures was bound to rows B, D, F, and H as nonspecific control. The microtiter plates were able to discriminate known positive and negative control serum from mice. Further studies are planned to determine the cross-reactivity of antisera to these recombinant proteins, the time course of waxing antibody development in mice after homologous nematode infestation, and the time course of antibody waning after treatment with anthelmintic.

## PS72 A New Method for Induction of Specific Immune Response in Mice by Oral Immunization with *Trichoplusia ni* Larvae Producing a Virus-Derived Antigen

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Infection of mice with viruses using natural or modified routes of infection is a common technique to induce pathogen-specific immune response. Due



to manipulation with live and often highly contagious viruses, this method could be associated with extreme risk of animal facility contamination. This contamination can be avoided, if the pathogen-specific antibodies are induced by inoculation of animals with virus derived recombinant proteins produced in different expression systems. However, since the latest approach requires application of highly purified proteins, the procedure can be complicated, expensive, and laborious. Here, we report a novel method for induction of the antigen-specific immune response by feeding mice with *Trichoplusia ni* larvae expressing the recombinant VP2 capsid protein from mouse minute virus (MMV). To generate *T. ni* larvae expressing MMV VP2, the corresponding gene was placed under transcriptional control of the baculovirus polyhedrin promoter that induced production of the VP2 protein in baculovirus-infected larvae. High levels of the VP2 expression in insect larvae (1.2 mg of the VP2 protein per 1 g of insect biomass) were verified by Western immunoblotting with MMV specific antibodies. Antigenic features of the VP2 protein produced in larvae were confirmed by corresponding ELISA. After consumption of larvae expressing MMV VP2, mice developed antigen specific immune response as determined by MMV specific ELISA and IFA. With this method we were able to get 71% of animals seroconverted, while a more common approach, in which animals were inoculated orally with purified MMV, resulted in only 36% of seroconversion. Thus, the described process provides significant time and cost savings, does not require purification of antigens, is less stressful to animals, and eliminates the risk of animal facility contamination. Moreover, the results of this study suggest the probability of using antigens produced in *T. ni* larvae as an oral vaccine candidates with minimal processing technology.

### PS73 Production of Human Lactoferrin in Transgenic Zebrafish

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Human lactoferrin (hLF) is a metal-binding glycoprotein involved in the host defense against infection and excessive inflammation. As the availability of natural hLF is limited, alternative means of production of this biopharmaceutical are extensively studied. While some data using variable bioreactors is compelling, reported fungi and yeast systems require expensive purification process and mammalian systems, including cows, harbor harmful mammalian disease-causing virus and microbes for production of hLF. To overcome those limitations, we have developed a transgenic zebrafish (*Danio rerio*) system with the vector construct carrying intact hLF-encoding cDNA, 2.1 kb. We obtained, by microinjection, transgenic zebrafish line 0801 harboring the genomic hLF gene under regulatory control of the zebrafish muscle specific promoter. The expression of hLF protein was confirmed by ELISA analysis and Western blot. Western blot analysis of recombinant hLF from transgenic zebrafish and natural hLF showed an 80 kDa-band. Expression level was about 3.2 µg/g of zebrafish tissue. Affinity interaction chromatography was used for hLF protein purification procedure with mouse monoclonal hLF antibody. The 3-dimensional structure of recombinant hLF closely matched the structure of natural hLF. Recombinant and natural hLF were equally effective in 2 different in vitro co-culture models, *Salmonella enterica* serovar Typhimurium (ATCC1311) and *Staphylococcus aureus* (ATCC 25923), by monitoring optical density at 630 nm. Taken together, the results illustrate the potential of the recombinant hLF from transgenic zebrafish in the production of biopharmaceuticals.

### PS74 IL-10 Deficiency Attenuates Gallstone Formation in a Susceptible Mouse Model

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We demonstrated that acquired immunity is instrumental in cholesterol gallstone formation. Mice lacking T-cells do not form cholesterol gallstones at an appreciable rate (<10%). However, transfer of functional T-lymphocytes

restores the phenotype. The current study expands upon these novel findings and analyzes the role of IL-10 and IL-4 in cholesterol gallstone pathogenesis. We compared male wild type C57BL/6J (B6) mice ( $n = 13$ ) with congenic IL-4<sup>-/-</sup> ( $n = 10$ ) and congenic IL-10<sup>-/-</sup> ( $n = 11$ ) mice. Beginning at 8 to 10 wk of age all mice were fed a lithogenic diet (1.0% cholesterol, 0.5% cholic acid, 15% triglyceride) for 10 wk. At the conclusion of the study, mice were euthanized with CO<sub>2</sub> and gallbladder bile phenotype was analyzed by microscopy. Normalized gallbladder weight ( $1.4 \pm 0.2$  mg/g) and mucin gel formation score ( $1.2 \pm 0.18$ ) were significantly ( $P < 0.05$ ) reduced in IL-10<sup>-/-</sup> mice compared to either IL-4<sup>-/-</sup> ( $2.2 \pm 0.9$  mg/g;  $2.2 \pm 0.8$ ) or B6 ( $2.4 \pm 0.3$  mg/g;  $2.5 \pm 0.9$ ) mice. IL-10<sup>-/-</sup> mice displayed a significantly reduced prevalence of cholesterol monohydrate crystals (55%) compared to B6 mice (92%,  $P < 0.05$ ) and a nonsignificant yet moderate reduction compared to IL-4<sup>-/-</sup> mice (90%,  $P = 0.15$ ). Finally, cholesterol gallstone prevalence was significantly reduced in IL-10<sup>-/-</sup> (36%) mice compared to either IL-4<sup>-/-</sup> or B6 mice (90% and 85% respectively,  $P < 0.05$ ). These data demonstrate that IL-10 deficiency appreciably reduces the prevalence of cholesterol gallstones as well as ameliorating the preliminary stages of cholelithogenesis (cholesterol monohydrate formation) in susceptible mouse strains. In contrast, IL-4 deficiency does not substantially alter cholelithogenesis in this mouse model. Since IL-10<sup>-/-</sup> mice displayed significantly reduced gallbladder volumes and mucin gel accumulation, a potential mechanism for this protection is repression of mucin glycoprotein production as well as maintenance of appropriate gallbladder wall contractility. These data starkly contrast with other models of chronic gastrointestinal inflammation in which IL-10 deficiency promotes disease.

### PS75 Lack of Serious Side Effects in Mice Treated with Doxycycline

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Doxycycline is used to regulate gene expression in inducible transgenic mice. Severe side effects of doxycycline would necessitate the investigation of high-priced alternatives. We evaluated the side effects of doxycycline in mice following their maintenance on medicated chow (100 mg doxycycline/kg or 1 g doxycycline/kg) or control chow for 7, 28, and 56 d. Animals were observed twice per week and weighted weekly. No negative side effects were visible. In fact, the low dose doxycycline produced a significant weight gain in the long term study, whereas high dose doxycycline had no significant effect ( $P = 0.02$ ). The ceca of doxycycline-treated mice were slightly bloated, which was observable after weighing of contents and statistical analysis (control, content 0.35 g, high dox 0.55 g,  $P < 0.0001$ , low dox, not significant). There was no effect of doxycycline treatment on the weight of the liver or kidneys. The abundance of symbiotic microbes in the gut was assessed by real-time PCR. Bacteria were reduced, but not eliminated, by doxycycline treatment. Stomach, duodenum, jejunum, ileum, cecum, colon, right kidney, and liver were embedded, sectioned, mounted, and stained with hematoxylin and eosin (H&E) for histopathologic evaluation. Minimal, multifocal, centrolobular, hepatocellular vacuolar degeneration, or microvesicular steatosis was occasionally observed within sections of the liver; however, these symptoms did not differ significantly among treatment groups. All other tissue sections evaluated were considered to be within normal limits with no indication of toxicological influence or tissue alteration initiated by the doxycycline exposure. The general side effects of doxycycline treatment are small and can easily be managed. However, in any experiment involving gene induction by doxycycline, a doxycycline-treated nontransgenic control must be included. Also, other methods of induction may be warranted if subtle effects on digestion and metabolism are studied as doxycycline alters the abundance of the intestinal flora and causes weight gain.

### PS76 Assessment of Pain and Distress Associated with Footpad Injections of Complete Freund Adjuvant in Lewis Rats

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Footpad injections of myelin basic protein (MBP) emulsified into complete Freund adjuvant (CFA) are used to induce experimental autoimmune encephalomyelitis (EAE) in Lewis rats, the animal model of multiple sclerosis. However, there are concerns about pain and distress associated with footpad injections. This study evaluated Lewis rats that were subjected to

different routes of injection with CFA. We hypothesized that stress-induced changes in behavior and physiologic parameters would be higher in rats immunized in the footpads as compared to those immunized at the tail base and nonimmunized controls, but that all immunization sites would be equivalent in inducing the intended disease. Telemetry transmitters were surgically implanted in the abdominal aortas of 54 Lewis rats to record body temperature, blood pressure, and activity. The rats were also videotaped for ethological assessment. Nine rats were randomly allocated to 1 of 6 different immunization treatment groups: 1) hind footpads with MBP/CFA; 2) front footpads with MBP/CFA; 3) tail base with MBP/CFA; 4) hind footpads with ovalbumin in CFA; 5) hind footpads with saline; and 6) no manipulation. Blood pressure, temperature, ethological profile, histopathology, and ability to induce EAE were compared between groups. Initial analysis of the physiologic results suggested elevations in blood pressure between groups 1 through 4 as compared to groups 5 and 6. Initial analysis of the ethologic data suggested that rats immunized in the footpads spent more time engaged in behaviors consistent with distress. Microscopic evaluation of the heart and intestines did not reveal significant fibrosis or inflammation in any of the treatment groups. Evaluation of the animal model (that is, time to onset of disease, lymphocyte proliferation) showed no significant differences between groups 1 through 3. We recommend that alternatives to the footpad injection site be used to minimize potential pain or distress for the animals.

### PS77 Postoperative Analgesia in a Mouse Mammary Cancer Model

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Current opinions regarding the use of animals in research necessitate that pain or distress be minimized; however, in certain circumstances there is a lack of literature to help guide investigator analgesic use for particular experimental models. Differences in opinion and personal attitudes regarding analgesic use for minor procedures in mice make it difficult for research facilities to set up guidelines. We determined the level of pain elicited by mammary fat pad removal surgery, a common minor experimental procedure used to study mammary gland tumor biology, and the effects of postoperative analgesics on recovery. Twenty-four 3 to 4-wk-old female FVB mice were anesthetized and had mammary gland removal surgery performed. Following surgery, mice were randomly placed in groups of 6 and assigned to receive carprofen, buprenorphine, a combination of carprofen and buprenorphine, or saline treatment. Six additional mice received anesthesia with no surgery or treatment. To evaluate postoperative pain, body weight, food and water intake, wheel running activity, and pain index scores were recorded daily for 4 d after surgery and compared to presurgical findings. Buprenorphine treatment and combination treatment significantly decreased food intake and body weight when compared to nonsurgical controls. All treatment groups had significantly decreased wheel running activity when compared to nonsurgical mice, and buprenorphine-treated mice had significantly lower wheel running activity when compared to carprofen-treated mice as well. Within treatment groups, pain index scores were lowest for carprofen-treated mice and highest for buprenorphine-treated mice through postoperative day 4. Based on these results, food and water intake, body weight, and wheel running activity did not indicate improved recovery with administration of analgesics when compared to the saline-treated group. However, the pain index scores suggest that mice treated with carprofen after mammary fat pad removal surgery recover more smoothly than untreated mice or mice treated with buprenorphine or buprenorphine-carprofen combination treatment.

### PS78 Swine H2N3 Influenza A Virus Pathogenesis in a Macaque Model

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Newly emerging influenza viruses with pandemic potential are of high public health concern, and the ability to study the pathogenesis of these viruses in an animal model is critical for the development of therapeutics and vaccines. We used a recently established nonhuman primate model to evaluate a newly emerged influenza A virus isolated from pigs. This virus was classified as swine H2N3 and had acquired the ability to bind to the human receptor, an important prerequisite for the generation of a new virus

that can readily infect and transmit between humans. *Cynomolgus* macaques were challenged with H2N3 as well as with a known H2N2 human influenza A for comparison. Animals were monitored at several time points over a 14-d period for virus growth, clinical symptoms, hematological parameters, radiographic changes, and for histopathologic evaluation upon necropsy. Hematological and clinical evaluation of the animals revealed minor changes over the course of the study. However, by day 3 postinfection (PI) there were evident radiographic changes in the lungs, which persisted over the 2-wk period in both groups. Virus was isolated from the lungs and several other tissues at day 1, 3, and 6 PI, which was indicative of a robust infection. Histopathology of hematoxylin and eosin (H&E) stained sections of lung revealed multifocal, suppurative pneumonia from day 1 samples, usually confined to 1 or few lobes. By day 6 PI lung samples were characterized by more extensive, moderate to severe pneumonia with abundant fibrin, edema, and type II pneumocyte hyperplasia. On day 14 PI lung samples revealed fibrosis with epithelial cell attenuation and metaplasia. Overall, the radiographic and histopathologic changes observed in H2N3-infected macaques were markedly more severe than in H2N2-infected animals. This study was able to evaluate multiple clinical parameters as well as virological and pathologic data in a newly established primate model. It will be of tremendous value for the study of existing and emerging highly virulent human influenza A viruses and their pathogenic potential.

### PS79 Multimodal Analgesia for Ruminants Undergoing Lateral Thoracotomy for Ventricular Assist Device Implantation

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Holstein calves (90 to 110 kg) and Dorset-Finn lambs (15 to 20 kg) are used as animal models for adult and pediatric left ventricular assist devices, respectively. The surgical approach involves entry into the thorax through the fifth intercostal space and creation of a subcutaneous pocket to house the pump. Successful chronic studies of up to 60 d necessitate rapid recovery, including a healthy appetite and return to standing within 24 h of surgery. Pain management that does not interfere with rumination is a crucial part of postoperative care. To that end, we have developed a plan that includes systemic opioid and nonsteroidal antiinflammatory drugs plus local analgesia delivered to the thoracotomy site via a wound diffusion catheter. One hour prior to surgery, animals receive flunixin meglumine (1 mg/kg IV). After intubation, intravenous fentanyl infusion (1 to 3 mcg/kg/h lambs, 5 to 8 mcg/kg/h calves) is begun and continued throughout surgery. Fentanyl (50 mcg lambs, 100 to 150 mcg calves) is bolused as needed in response to increases in heart rate or blood pressure. During closure of the muscle layers of the thoracotomy incision, a wound diffusion catheter is placed, into which bupivacaine (12.5 mg lambs, 20 mg calves) is instilled. Fentanyl is discontinued, and after a 10 min washout period, buprenorphine (0.005 mg/kg IV lambs and calves) is administered. Flunixin is repeated prior to ending inhalant anesthesia. In the postoperative period, analgesics are continued as follows: 1) flunixin 1mg/kg IV q 12 h; 2) buprenorphine 0.005 mg/kg IV q 8 h; 3) bupivacaine 12.5 mg lambs, 20 mg calves in wound catheter q 8 h. The wound catheter is removed after 72 h. Buprenorphine is discontinued in the following 24 h, followed by flunixin. Animals are closely monitored 24 h per day for signs of pain, including bruxism, kicking, rolling, inappetence, and tenderness at incision sites. Rumen motility is also carefully assessed. Using this approach, calves and lambs stand and eat within 24 h after surgery, have no appreciable decrease in rumen motility, and show minimal signs of pain and discomfort.

### PS80 A Novel Method to Establish a New Specific Pathogen-Free (SPF) Laboratory Rabbit Strain with Pigmented Eyes

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Rabbit strains with pigmented eyes offer advantages for ocular drug development and research over the unpigmented eyes of albino strains because the melanin in the eyes may react differently with certain drugs. However, specific pathogen-free (SPF) strains of rabbits with pigmented eyes, other than black Dutch Belted (DBs), bred exclusively for research are uncommon. A new rabbit strain, created from crossing SPF New Zealand whites (NZWs), strain designation Hra: [NZW]SPF, and New Zealand reds (NZRs),

was desired for the characteristics of pigmented eyes and a larger body size as compared to DBs. To pursue development of this new cross, NZRs were obtained from a hobby breeder source. Utilizing a novel method combining herd health management, quarantine housing, preemptive treatments, targeted treatments, sentinel animal diagnostics, and serial diagnostics on future breeder animals, the NZRs were successfully converted to an acceptable SPF status for research purposes. Treatments and diagnostics were aimed at bacterial pathogens (*Pasteurella multocida*, *Pasteurella pneumotropica*, *Salmonella*, *Clostridium piliforme*, *Treponema cuniculi*, and *CAR Bacillus*), protozoa (*Encephalitozoon cuniculi*, *Toxoplasma gondii*, and *Eimeria* spp.), dermatophytes, ectoparasites (fur mites, ear mites, lice, and fleas), and endoparasites (cestodes and nematodes). This method eliminated the need for cesarean rederivations, which would have increased the number of animals needed, and artificial insemination, which would have introduced disease transmission risks. It expedited the ability to breed the animals at an acceptable SPF health status and decreased the overall number of animals needed for breeding stock.

#### PS81 Withdrawn

#### PS82 Soaking Surgical Instruments and Gloves in Isopropyl Alcohol between Serial Mouse Laparotomy Surgeries Is Effective

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Applying the principles of aseptic surgical technique to rodent surgeries performed in series presents many challenges to the principle investigator, the attending veterinarian, and the IACUC. We examined the effectiveness of 70% isopropyl alcohol soaking on bacterial decontamination of surgical instruments and gloves used in a series of rodent surgeries. If effective, we also wanted to determine at which point in the series of 10 surgeries fresh instruments and gloves should be obtained. This study examined and tested our own university's guidelines for aseptic recovery surgery performed in rodents, particularly mice, as it relates to surgical instrument and glove contamination. Thirty-five ICR mice (*Mus musculus*) underwent a terminal subcapsular renal injection surgical procedure. The mice were divided into 1 control group ( $n = 5$ ) and 3 experimental groups (I, II, III where  $n = 10$ /group). The control group used autoclaved instruments and sterile surgical gloves for each mouse surgery. Since we wanted to know at which point in a series of 10 surgeries alcohol soaks would no longer be effective at decontaminating our instruments and gloves, we had 3 identical groups of  $n = 10$ . Each experimental group began with autoclaved instruments and sterile gloves. Instruments and gloves were soaked in 70% isopropyl alcohol between each mouse surgery for all experimental groups. Cultures of instruments and glove fingertips were taken before (autoclaved or post-alcohol soak) and after the surgical procedure for each mouse. Results were classified as either bacterial "growth" or "no growth" and treated as binary data with  $\alpha = 0.05$ . Bacterial growth on surgical instruments was noted in 2 experimental groups after 5 surgeries. We reasonably concluded that the modified aseptic technique using 70% isopropyl alcohol soaks for instruments and gloves up to 5 surgeries is safe in most cases.

#### PS83 Alopecia in Macaques at a Research Facility: Patterns, Prevalence, and Partners

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Alopecia in research macaques is considered a potential stress indicator by some laboratory animal experts; however, most published reports about hair loss assessed group-housed monkeys that were minimally involved in biomedical research. A study was undertaken to characterize patterns of alopecia at a nonhuman primate facility devoted to cognitive neuroscience research. Over half of the animals in the survey were pair-housed versus living alone, and the colony was comprised of approximately twice as many males (55) as females (27). Dorsal and ventral views of the nonhuman primates were photographed during quarterly physical exams over a 2-y period. The extremities and dorsum, areas noted to be most affected by alopecia, were divided up anatomically, for example, carpus, forearm,

elbow, upper arm. Hair loss scores were assigned to each body part; in addition, the total number of affected body parts was taken into account. Throughout the study period, extent and severity of alopecia did not differ significantly regardless of pairing status or sex ( $P$  values ranged from 0.2 to 0.87), and the majority of animals (88%) had at least mild, focal hair loss on 1 occasion. Instances of missing hair were also compared to individual characteristics such as age, body condition score, and abnormal behaviors, as well as to external factors for an animal at each timepoint, including use by the research laboratory and surgical episodes. Results from the survey define common sites of hair loss in this population, chronicle changes over time in each animal, and allow insight into the occurrence of excessive autogrooming and allogrooming.

#### PS84 A Psychologic Wellbeing Response Plan for Nonhuman Primates

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Under animal care situations that conform to compliance standards for environmental enrichment established by the Animal Welfare Act (AWA) and the *Guide to the Care and Use of Laboratory Animals*, at least 10% of laboratory macaques exhibit abnormal behavior, with 1 study reporting 1 or more abnormal behavior in 321 of 362 monkeys (89%). The AWA also requires that nonhuman primates (NHPs) exhibiting signs of psychologic distress be "provided special attention regarding enhancement of their environment." We devised a behavioral response plan for NHPs that present commonly recognized abnormal behaviors compatible with psychologic distress (that is, self-directed behaviors in which the animal directs aggressive or unexplained attention towards its body, self-abusive behaviors that result in trauma, repetitious locomotive behaviors, hair pulling or plucking resulting in alopecia, hyperactivity, and fear and aggression; fear and aggression were considered abnormal behavior because they imply the animal was adversely affected by its environment or personnel, and that intervention was indicated). The plan included specific recommendations regarding intervention, frequency of care, personnel responsible for care, documentation, and follow-up evaluation. Animal care personnel were trained to identify the abnormal behavior in order to immediately implement a preliminary intervention before a formal assessment could be made by veterinary or behavioral staff. Interventions were standardized and based on processes that had eliminated or reduced the specific behavioral problem in past cases. This plan eliminated abnormal behaviors in 21 of 27 (78%) macaques within 8 wk of treatment initiation, with subsequent elimination in 3 more by 16 wk, leaving only 3 cases (11%) unresolved. Resolution of approximately 90% of cases confirms the benefit of a standardized plan for commonly recognized abnormal behaviors in the majority of laboratory NHPs. Refractory cases confirm the need for continued investigation of abnormal behaviors in captive animals.

#### PS85 Benefit of Decreasing Noise Stimulation for Anesthetized Nonhuman Primates Undergoing Magnetic Resonance Imaging Scans

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Magnetic resonance imaging (MRI) is widely performed on various laboratory animal species. Most MRI procedures require several imaging sessions, with a typical functional brain anatomy scan including 3 to 9 sequences and minimum duration of 60 min. Magnetic field alterations created during scans are not felt physically by human subjects, so no pain to a conscious or immobilized animal is normally expected. However, loud acoustic noise (around 118 dB(A) in many 3Tesla magnets) is generated during normal use and human subjects are routinely offered earplugs, headphones, televisions, or music to alleviate discomfort. It was noticed that anesthetized nonhuman primates exhibited increased heart and respiration rates when scanning noises commenced. The objective of this study was to assess animal welfare benefits of noise reduction during MRI scans involving eight 3.5-y-old rhesus macaques. Animals were provided routine veterinary support in the course of general anesthesia during 4 separate scans of 90 min each without any noise reduction, to establish a baseline. Two groups of 3 animals were provided with either pediatric earplugs or ear-muff-style pneumatic headphones playing classical music at 60 to 80 dB during an additional 4 scans



each; the other 2 animals were evaluated with both earplugs or headphones during 2 scans each. Heart and respiratory rates, anesthetic requirements to maintain immobility, and image quality (as a function of immobility) were monitored from anesthesia start through recovery, with attention paid to differences in rate peaks during different noise attenuations. Degrees of noise reduction by each method were based on manufacturers' claims. Although both methods, versus baseline values, resulted in statistically significant ( $P < 0.05$ ) benefits, headphone use was better, resulting in 30% less anesthetic, 18% lower heart and respiratory rates during known loud sequences, and improved recovery times by over 10 min. Both noise reduction devices were easy to use and no adverse effects were observed.

#### PS86 A Novel Warming Device for Animal Anesthesia Recovery

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The recovery period after anesthesia is critical for minimizing body heat loss in laboratory animals in order to avoid stress and discomfort. Depending on the ambient temperature of the room, the body temperature of the animal, and the duration of recovery, various devices, such as heat lamps, heating pads, or warm water bottles, may be indicated. However, hot water bottles do not conform to the animal's torso and may obstruct normal breathing if placed incorrectly. Also, heat lamps and electrical heating pads may cause superficial burns or overheating, and circulating water heating pads can be easily torn and leak. Furthermore, postanesthesia care must ensure personnel safety during recovery and handling of the animal. A novel recovery jacket was developed that maintained supplemental heat for 40 min or more at 102.4 °F. The jacket, a modified human foot brace with fleece lining and microwavable rice pouches for heat retention, was evaluated on 6 macaques anesthetized while undergoing fMRI brain scans. Variables measured included core body temperature at end of anesthesia and upon returning to the home cage as well as level of spontaneous activity (degree of recovery from anesthesia) determined by visual observations. Data were compared to 3 macaques recovering with a hot water bottle starting at 100 to 102 °F and 3 macaques recovering with a heat lamp positioned 12 in. from the cage providing variations of 76 to 110 °F at body surfaces. In all 6 animals using the jacket, core body temperatures were 1 to 2 °F warmer versus bottle or lamp. Mental stimulation was provided as animals easily slipped out of it and played with it during recovery. There were no adverse effects observed and no personnel safety risks in using this device. In addition, this device may be modified for other species by manipulating its shape and size.

#### PS87 An Unusual SRV/D-T, SRV-4-Like Outbreak in Cynomolgus Monkeys in the United States

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The simian retrovirus type D (SRV/D) belongs to the betaretrovirus genus and exists as both an endogenous or exogenous form in nonhuman primates. Disease signs vary from no clinical symptoms to fatal immune deficiency. At least 5 serotypes of exogenous SRV/D have been reported to date, but only SRV-1, 2, and 3 whole genomes have been fully sequenced. SRV-4 was recovered once from a group of cynomolgus macaques in California in the middle of the 1980s, but no genome sequences had ever been published. In Japan, a new SRV subtype SRV/D-Tsukuba (SRV/D-T) was isolated from cynomolgus macaques in 2005. Here we report a SRV-4-like outbreak in the United States (US) of a virus that has been found to be phylogenetically close to SRV/D-T virus. Based on preliminary sequencing data of the partial *env* gene of a SRV-4 positive control DNA, a total of 42 young cynomolgus monkeys that were imported from China to the US from December 2008 to May 2009 were found to harbor SRV/D DNA with high homology to the SRV-4. The virus was isolated (D/CYN/TX) and its whole genome sequenced (8,126 base pairs). Molecular genetic data showed a typical SRV/D viral genome organization (5' LTR-gag-prt-pol-env-3' LTR) with 78.3%, 75.5%, and 74.3% homology to SRV-1, 2, and 3 respectively. Phylogenetic analysis demonstrated that D/CYN/TX isolate was distantly related to SRV1, 2, 3, 5, 6, and 7, but

it was clustered together with SRV/D-T. Serological tests indicated that antibodies from infected animals not only cross-reacted with SRV-2, but also with SRV-1 and SRV-5. Complete gross necropsy and histologic assessment showed that no significant pathologic changes had been associated with this SRV/D-T, SRV-4-like virus. These results reveal either the emergence or the undetected presence of SRV/D-T, SRV-4-like virus in the US. The genetic variation observed in D/CYN/TX compared to other SRV/D could be due to the evolutionary divergence related to the different geographic origin.

#### PS88 Withdrawn

#### PS89 Quarantine Procedures and Health Assessment for California Ground Squirrels (*Spermophilus beecheyi*)

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California ground squirrels (CGSs), *Spermophilus beecheyi*, are highly visual rodents and are being used to examine the neural basis for visual behavior and visual memory with the goal of developing an alternative to the use of nonhuman primates in visual neurobiologic research. Our institution planned to acquire young adult CGS born from wild-caught, pregnant dams from another institution. We reviewed the literature and consulted with the staff at the institution of origin, the Centers for Disease Control (CDC), wildlife veterinarians, and our institutional biosafety committee to develop a list of infectious agents that may infect CGSs, some of which are zoonotic. Following a risk assessment, the agents were prioritized, associated testing methods and testing laboratories were identified, and occupational health and safety procedures were defined. The CGSs tested negative by serology at the CDC for *Yersinia pestis* and *Francisella tularensis* prior to receipt. The CGSs were housed in microisolation cages in quarantine at ABSL-2 upon receipt. Health assessment included physical and ocular examination, microscopic examination of skin scrapes, skin and anal tapes, rectal bacterial culture including *Salmonella*, *Shigella*, and *Campylobacter*, examination for ova and parasites by fecal flotation and centrifugation, *Cryptosporidium* and *Giardia* testing by fecal ELISA, and *Helicobacter* by fecal PCR. Mouse and rat sentinels were exposed weekly to dirty bedding from the CGS cages for 8 wk and then submitted for comprehensive health monitoring. The CGS tested positive for *Septhacia* spp., *Aspiculuris tetraptera*, *Eimeria*, *Entamoeba*, *Giardia*, *Chilomastix*, and *Helicobacter*. The CGSs were treated with fenbendazole, metronidazole, and sulfadimethoxine in apple- or grape-flavored tablets to eliminate organisms other than *Helicobacter*. Despite repeated treatments with metronidazole and fenbendazole, some of the CGSs remained positive for *Giardia*. Mice and rat sentinels tested negative for all agents on comprehensive health monitoring.

#### PS90 Transforming Classroom Demonstrations into Efficient and Effective Media Presentations

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As teachers and trainers, we all face the challenge of accurately, effectively, and efficiently demonstrating hands-on procedural techniques in a classroom setting. Simply standing next to a bench and performing restraint, bleeding, injections, and other techniques has several limitations. In particular, some students may find it difficult to see the demonstration, and the instructor may not always perform the procedure consistently. We describe our development of a highly effective training program that combines hands-on demonstration, digital photographs, handouts, and video clips. These tools are used to prepare students for supervised classroom practice of each technique, and require nothing beyond a basic digital video camera and commercial software. We demonstrate both our finished training program and the steps others could follow to develop a program suited to their specific need.

#### PS91 Cost Efficiency of Ventilated Rodent Housing in the Conventional Environment

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Most facilities view the use of ventilated housing only in a barrier environment. However, with the recent downturn of grant renewals, facility managers and supervisors are asked to do more with fewer funds. Furthermore, in an effort to save grants funds, investigators at my institution have asked to transfer many of their barrier mice into conventional housing. This caused an excess of ventilated racks not being used. Continuing the use of ventilated racks in a conventional environment instead of transferring them into regular shoebox cages actually saved the department on labor and material cost. We compared costs of bedding, detergent, labor, and staff numbers for 1,000 open shoebox conventional mouse cages with 1,000 similar size ventilated cages. Results showed an overall annual cost savings of US\$32,610.24 when animals were housed in ventilated caging.

### **PS92 Using Vaporized Hydrogen Peroxide to Sterilize Supplies when Bulk Autoclaving Is Not an Option**

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On September 12 2008, Galveston was directly hit by Hurricane Ike. Recovering from Ike has been difficult due to the loss of critical equipment caused by water damage. Normal business has continued for our researchers, but some of our normal procedures have been modified. The start of a new breeding and sentinel colony prompted the need for autoclaved supplies; however, bulk autoclaves were lost due to water damage. Utilizing a vaporized hydrogen peroxide (VHP) generator to sterilize ventilated racks and bulk caging became a feasible option. An empty room was designated and prepared with the VHP and aeration unit. A ventilated rack filled with complete cages (minus bedding) were placed into the room and connected to an air supply unit. While the rack was operating, the VHP machine was run on a medium load cycle. Upon cycle completion, the rack and air supply unit were transported to the appropriate room. Preparing bulk caging for change-outs was performed by placing empty crisscross-stacked cage bottoms, microisolation tops, and wire lids into a mobile shelving unit (MSU). The MSU was placed into the designated VHP room and the machine was run on a heavy load cycle. Upon cycle completion, cages were bedded with autoclaved bedding, placed back in the MSU, covered, and moved into a room requiring the supplies. Biologic indicators were placed within the cages in the rack, the crisscross-stacked cages, microisolation tops, and wire lids. Supplies were confirmed as "clean" by using biologic indicators, ATP testing, plating wipe samples, and mouse serology reports. This method has reduced both autoclave run time and cage deterioration. The cost for VHP supplies was less than US\$20 per cycle.

### **PS93 Leadership Practices in Laboratory Animal Medicine**

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Many laboratory animal medicine department activities are provided as services to scientific investigators, and as such may sometimes be taken for granted. Motivating staff who work in animal resource departments can be achieved by aligning good communication, clear goals and rewards, and opportunities to improve skills and knowledge. Successful leaders provide a compelling picture or vision of the future and outline steps to move toward that future. Strong leaders are able to increase productivity, defined as results delivered divided by resources used, by increasing employee engagement, which can be measured using employee engagement surveys or other tools. In order to improve productivity, we used findings from the employee engagement survey questions to intentionally improve engagement, and specific actions were initiated to increase communication, recognize accomplishments more often and more publicly, and create more learning opportunities for personnel. Increased participation in scientific studies led to an improved reputation outside the department. To support increased communication, a multimedia approach was used including video, web messages, articles about colleagues and events, small group meetings, and large department meetings. Recognition was improved using written messages (email and handwritten), announcements during department meetings, and judicious use of cash awards. Employees at all levels were encouraged to increase skills and support partners through rotations and volunteering to help with scientific procedures, short term travel to other locations, online courses, didactic sessions, and global interactions with peers. Employee engagement was measured in 2007 and 2008 using the employee engagement survey. The leadership initiatives, paired with

an understanding of the essence of value delivered, resulted in improved employee engagement, emergence of new leaders in the department, and enhanced delivery of services to customers.

### **PS94 A Comprehensive Laboratory Animal Program Pandemic Response Plan**

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The potential of an influenza pandemic calls for the development of an organized, rational plan for continued laboratory animal facility operation without compromising the wellbeing of employees and animals. We created a comprehensive laboratory animal program pandemic response plan as an integrated component of the university-wide response. Preparation involved all levels of organizational hierarchy, including the IACUC. Many contingencies and scenarios were considered to create a step-by-step plan based on the World Health Organization's (WHO) phase alert criteria and federal and local health departments' recommendations. The most extreme situation model requires suspending research operations and maintaining reduced numbers of laboratory animal colonies and personnel for up to 6 mo. For this plan and for lower levels of preparation, features included active recruitment and cross training of volunteers for essential personnel positions, protective measures for employee and family health, logistical arrangements for delivery and storage of food and bedding, the removal of waste, and the procedures for euthanasia of large percentages of the animal census. Strategies, such as a mouse cryopreservation initiative, were implemented to minimize potential loss of valuable research and to decrease facility restart time. Elements of this plan were put into practice following escalation of the WHO pandemic alerts due to Influenza H1N1 in April 2009.

### **PS95 Life Cycle Analysis of an Individually Ventilated Cage Rack: From Cradle to a New Cradle**

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Making informed decisions about the environmental impact of a product requires factual, empirical data and not hype or supposition. This study is a continuation of a previous one in which the CO<sub>2</sub> emissions of an individually ventilated cage (IVC) were evaluated using life cycle analysis (LCA), that is, a scientific methodology used for assessing the environmental impact during the product life cycle. The current study complements the previous one by evaluating the CO<sub>2</sub> emissions of the stainless steel IVC rack, which, together with the cages, forms the complete IVC rack. The same LCA technique has been employed using realistic and reliable data collected from suppliers of raw materials, intermediates, and components, including all packaging and transport, production and distribution, use and disposal of the IVC rack. The output of the study focuses on energy consumption and carbon footprint contributions from green house gases (GHG) emissions with data and results shown in terms of direct inventory data, such as energy use and GHG emissions. Although the study demonstrates a low level of GHG emissions, a sensitivity analysis was performed evaluating the IVC rack design for possible ways to further reduce environmental impact during production (reducing material and weldings) and increase the ease with which the product can be disassembled at the end of its life for increase recycling, moving beyond "cradle to grave" toward the new paradigm of industrial ecology "from cradle to a new cradle." Reducing material and energy consumption and increasing the recycling of material we reduce the carbon footprint of products according to the country emission factor, that is, the amount of CO<sub>2</sub> emission per energy unit (in Italy, 0.54 kg of CO<sub>2</sub>/kWh). Through the use of LCA, the laboratory animal professional, aware of their social responsibility to the environment, can now make informed choices about how to reduce the carbon footprint of their operations while also reducing operating costs.

### **PS96 Implementing a Medical Surveillance Program for Animal Care Staff**

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There are many reasons to implement medical surveillance programs for our animal care staff. We discuss the regulatory requirements that drive the need for these programs and the benefits and challenges to implementing a program in a laboratory animal facility. These programs also provide employees a way to voice any occupational or health matters before they reach a critical mass and a serious problem arises. Surveillance programs allow for early identification of animal-acquired allergies. There is also the added benefit of identification of nonwork-related health issues that can be an advantage to the employee and improve employee morale. We have implemented a program that begins with the employee job description and the new hire process. All employees who must enter or handle any animals as part of their job duties are required to have a post-offer, pre-employment physical evaluation. The employee completes a medical questionnaire, which is reviewed by the occupational health physician along with the employee. Any potential allergies or preexisting conditions that may prevent the employee from doing their job are discussed. Vaccines are also required as a condition of employment and are administered at this time. Furthermore, we require all our employees to wear PPE that includes outer garments, gloves, and N-95 respirators in animal rooms. This is done to prevent the development of LAAs. Nonetheless, when these are developed, we try to work with the employee by either placing them in additional PPE, such as powered air purifying respirators, or find them positions outside the animal laboratories, if available. Employees must also receive annual medical evaluations and may also be evaluated when there is an on-the-job injury, or any time they have a concern they wish to discuss with our physician. This program is administered by our staff through the Compliance and Security Office. We use an offsite occupational health clinic.

#### **PS97 Reducing Energy Use in Laboratory Animal Facilities while Potentially Improving Air Quality**

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The design of HVAC systems serving animal facilities is critical to the maintenance of the indoor environments suitable for animals and caregivers. Proper ventilation will reduce buildup of odors, allergens, temperature stratification, and metabolically generated gases. Reducing the ventilation air change rate can result in energy savings; however, indoor air quality may become compromised. The approach to the design of the ventilation system should strike a balance between installation and operating cost and indoor air quality to arrive at the optimum design criteria. This presentation will focus on design concepts and the numerical analysis of ventilation systems for a multispecies facility where a portion of the holding areas were renovated and others were newly constructed. Candidate designs for the HVAC systems were evaluated using computational fluid dynamics (CFD) to assess the rooms' odor levels, temperature uniformities, and general ventilation effectiveness. The subject facility achieved more than 50% savings in annual HVAC cost and initial installation costs, while also achieving virtually undetectable ammonia levels within the holding rooms. The designs of the new construction and the renovated rooms were challenging with constrained room, overhead, and lateral spaces. Additionally, the same type of caging, ventilated or otherwise, could not be used throughout. Therefore, the levels of odor, temperature, and allergens had to be controlled at the room level rather than from the cage level. We know that simply providing a certain number of air changes to a room does not necessarily result in good air quality; we must "tune" the ventilation system to the best distribution in order to provide the most effective use of fresh air. The savings are also calculated and presented for various regions of the country so the audience may gauge how these same techniques could impact their facilities. In addition, an enhanced procedure to quantify air exchange effectiveness will be presented that uses CFD to compare the efficiency of a perfect mixing case to the room design at hand. Some discussion will also cover various techniques, such as facility monitoring systems and digital controls, to further improve energy efficiency without degrading indoor air quality.

#### **PS98 Dyspnea and Lingual Swelling in a One-Year-Old Male Sprague-Dawley Rat**

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A 1-y-old male, experimentally naive, Sprague-Dawley rat presented for necropsy with a 1 d history of dyspnea. On examination there were bilateral cataracts and both corneas appeared roughened. The ventral cervical region and the distal aspect of the tongue were both swollen, causing the latter to protrude from the oral cavity. On necropsy, the prostate was severely enlarged, firm and nodular, the urinary bladder was severely distended with a dark brown-red fluid and a partial thickness tear was visible, there was bilateral hydronephrosis, and bilateral ureteral dilatations were noted. Microscopically bilateral hypermature cataracts, lens rupture, mild suppurative anterior uveitis, and acute ulcerative keratitis were observed. Severe edema secondary to a necrotizing vasculopathy was observed in the ventral cervical and lingual tissues. Necrotizing vascular lesions were also observed in the pancreas and small intestine. Urogenital tract lesions included bilateral hydroureter, a focal tear in the tunica muscularis of the bladder, a poorly differentiated prostatic adenocarcinoma and suppurative prostatitis, and vesiculitis. In both kidneys, glomerular, interstitial, and tubular degenerative and inflammatory changes consistent with chronic progressive nephropathy and a mild neutrophilic pyelonephritis were observed. Testicles were bilaterally atrophied and there was an absence of spermatogenesis. Soft tissue mineralization was observed in the aorta, stomach, and kidneys. A chief cell adenoma was detected in the left thyroid gland. Additional incidental lesions included myocardial degeneration and fibrosis, and pancreatic atrophy. The prostatic adenocarcinoma, an uncommon tumor of rats, resulted in urinary outflow obstruction and associated urogenital tract lesions. The necrotizing vasculopathy and soft tissue mineralization suggest uremia. Chief cell adenomas are rare in rats and may have contributed to metastatic calcification. Other incidental lesions noted in this case are associated with aging and occur with various frequencies in Sprague-Dawley rats.

#### **PS99 Coagulation Factor VII Deficiency in a Research Beagle Colony: Impact of a Molecular Genetic Screening Program**

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Coagulation Factor VII (FVII) deficiency is an autosomal recessive disorder known to exist within research beagle colonies since the 1960s. Dogs affected by this hereditary coagulopathy may show mild to moderate hemorrhagic tendency induced by trauma or surgery but are often incidentally discovered with only a mildly prolonged prothrombin time and normal partial thromboplastin time. Factor VII deficiency is caused by a missense mutation resulting in less than 5% plasma FVII activity. In late 2005 we initiated a FVII screening program within our beagle production colony using a mutation-based DNA test. Our primary goal was to remove all affected and carrier stud dogs from the breeding population so that no affected progeny would be produced. Secondly, we began screening all potential broodstock animals. Of the 2081 production and potential broodstock beagles screened through 2008, 76.3% were normal, 22.8% were carriers, and 0.9% were affected. Affected dogs had prolonged prothrombin times but they did not show any overt clinical signs while in our colony. Utilizing this screening program we successfully removed all affected and carrier stud dogs from the broodstock colony within 2 y of initiating the screening program. This effectively eliminated any production of FVII deficient offspring. Moreover, this status has been maintained through continued screening of all potential broodstock animals to further reduce the mutant allele frequency. We have thereby effectively refined our research canine model through implementation of a comprehensive genetic screening program. Such refinement of our canine model will minimize the effect a coagulopathy could have on pharmacotoxicological and other research studies.

### **Poster Sessions**

#### **P1 The Use of Postsurgical Wraps in a Pharmacokinetic Colony of Ferrets with Vascular Access Ports**

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Vascular access ports can be used in ferrets for pharmacokinetic studies as an alternative to repeated venipuncture. Unfortunately, ferrets have a tendency



to pick and chew at postsurgical incision sites. Given their highly curious nature and their high degree of flexibility, protecting the femoral incision site poses a challenge. Due to there being no commercially available products on the market made for this purpose, a creative solution was required that was economical, easy to use, and well-tolerated by the ferret. Using minimal supplies that included vet wrap, duct tape, and discarded radiograph film, a wrap was devised that limited the ferrets' access to the femoral area by reducing their flexibility. With only minor modifications to their living environment, the ferrets were still able to freely move around the cage. The post surgical wrap is placed on the ferret immediately upon recovery from anesthesia and is maintained for at least 1 wk after surgery before reevaluation. The ferret is then allowed a day of observation to determine if a longer period of time is needed for incision healing. If the ferret does not show signs of chewing, the wrap is not replaced and the ferret is allowed to heal on its own. If signs of chewing are still present, the ferret wrap is reapplied for another week. In both instances, the ferrets are monitored closely by the veterinary staff on a daily basis. This allows the animal to heal properly and reduces the need for surgical repair. Other observed advantages of using this system are minimized infection rate, faster assignment to study, and generally quicker recoveries. We have observed, however, that there is an average temporary weight loss of 15% concomitant with the wrap application as compared to an average weight loss of 7% in nonwrap ferrets. This typically reversed itself 2 to 3 wk after the wrap was removed. We feel the listed advantages vastly outweigh this 1 disadvantage. In addition to being economical, the ferret wrap is reusable and made of repurposed materials, making it a "green" solution as well.

## **P2 Pharmacokinetics of Enrofloxacin in African Clawed Frogs (*Xenopus laevis*)**

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In this pharmacokinetic study, approximately 100 sexually mature adult female *Xenopus laevis* frogs (approximately 4 y old, body weight 112 to 175 g, 10 to 12 cm SVL) were used for injection with the fluoroquinolone antibiotic enrofloxacin. The frogs were divided into 2 experimental groups of 50 animals. One group received intramuscular injections of enrofloxacin (10 mg/kg) into the left quadriceps muscle. The second group received enrofloxacin (10 mg/kg) as a subcutaneous injection in the left quadriceps region. Prior to injections, animals were further divided into subgroups of 4 and assigned 1 of the following time points: 0 min (pretreatment group), 10 min, 20 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h, 24 h, and 48 h. Animals in each subgroup were euthanized and blood was collected via cardiocentesis. Blood plasma was then analyzed for antibiotic concentration levels using high performance liquid chromatography (HPLC). From this data, the following pharmacokinetic parameters were computed using a noncompartmental analysis of the pooled concentrations (naive pooled samples). For intramuscular samples the half life ( $t_{1/2}$ ) was 5.32, concentration max of 10.85  $\mu\text{g}/\text{mL}$ , the distribution volume ( $V_d$ ) was 841.96 mL/kg, areas under the curve (AUC) 57.59  $\mu\text{g h}/\text{mL}$ , and mean residence time was 8.0 h. For subcutaneous samples the half life ( $t_{1/2}$ ) was 4.08, concentration max of 9.76  $\mu\text{g}/\text{mL}$ , the distribution volume ( $V_d$ ) was 915.85 mL/kg, areas under the curve (AUC) 47.42  $\mu\text{g h}/\text{mL}$ , and mean residence time was 6.1 h. Based on plasma pharmacokinetics, *Xenopus* appear to absorb enrofloxacin effectively given intramuscularly or subcutaneously. Concentrations reached high levels in plasma with no apparent histologic effects seen on histology after single dose administration.

## **P3 Technique for Footpad/Lateral Tarsal Region Injections in Mice**

AA Gyles\*

SoBran, NIAMS, Bethesda, MD

We provide precise details on how to prepare and administer injections into the tarsal region of the foot in mice. The tarsal region injection is an effective alternate route of inoculation for the classic method of injecting the footpad. The tarsal region injection site has the same physiologic result as injecting directly into the footpad, with the benefit of decreased swelling to the foot itself; therefore, reducing pain and distress to the animal. Utilizing the footpad for immunization of small rodents may be necessary in particular studies where the isolation of a draining lymph node, as a primary action site, is required. The wellbeing of subject animals is commonly addressed by limiting the quantity of adjuvant-antigen solution injected into the footpad and housing on soft

bedding. In instances where no specific justification is provided for footpad inoculation, this technique should not be used for routine immunization of rodents. Lateral tarsal region injection is an excellent alternative method for immunization in mice, especially to induce lymphatic drainage. This technique is easy and quick to master. In addition, anesthesia is not required for this type of injection. Post injection observations are required due to the potential for pain, distress, swelling, or infection. Post injection animals are placed on soft bedding and monitored daily for pain and distress.

## **P4 Hematological and Serum Chemistry Reference Values for Familial Hypercholesterolemic Swine**

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Familial hypercholesterolemia (FH) in swine is a complex lipid and lipoprotein disorder associated with development of severe coronary artery pathology similar to the lesions observed in human coronary artery disease. The purpose of the current study was to establish hematological and serum chemistry reference values for FH swine to better support clinical and research assessments. In this study, hematological and serum chemistry data were acquired from more than 50 healthy FH pigs (castrated males and intact females) ranging in age from 6 to 12 mo. Blood samples were collected using standard techniques in anesthetized animals. Whole blood and serum samples were submitted to a commercial clinical diagnostic laboratory for analysis. For each animal, a complete blood count with differential and a serum chemistry profile (22 parameters) was generated. Data was analyzed utilizing a commercially available statistical software package. Hematological and serum chemistry values were calculated including mean, standard deviation, and a 95% confidence interval. The FH swine data were compared to existing reference values for domestic swine and the data was analyzed with respect to both potential age and sex differences. Serum cholesterol and triglyceride levels were significantly elevated ( $P < 0.05$ ) for the FH swine compared to age matched domestic swine. For FH swine, serum cholesterol and triglyceride levels ranged from 393 to 439 mg/dL and 48 to 70 mg/dL, respectively. No other significant differences were noted for the hematological and serum chemistry values of FH swine compared to domestic swine. There were no significant differences in any parameter with respect to age or sex within the FH cohort. The establishment of reference values for the FH swine serves as a clinical resource, and will help facilitate the design of preclinical studies directed at the evaluation of new therapies for the treatment of coronary artery disease.

## **P5 Evaluation of Route of Administration and Dosage of Tramadol as an Analgesic in the Rat**

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Pain management is an important aspect of laboratory animal medicine and represents refinement as 1 of the 3 Rs. Successful analgesia in research animals directly impacts animal welfare and improves the quality of scientific data. Tramadol is an approved, opioid-like analgesic used in human and veterinary medicine to manage acute and chronic pain. A pilot study was initiated using 8-wk-old, male Sprague-Dawley rats to evaluate the optimum dosage and route of administration of tramadol (4 rats per treatment group). The 3 routes of administration evaluated were oral (PO) via a jello cube, intraperitoneal (IP), and subcutaneous (SC). The 5 dosage groups included 0 mg/kg (control), 4.0 mg/kg, 12.5 mg/kg, 25 mg/kg, and 50 mg/kg. Tramadol administered at 12.5 mg/kg IP increased the latency response in hotplate and tail flick testing. The subcutaneous group also demonstrated long-lasting analgesia at both the 12.5 and 50 mg/kg dosages; however, 3 of 4 rats developed skin lesions at the injection site 2 d post administration. The oral group demonstrated analgesia only in the initial 30 min in the hotplate test, but then decreased possibly due to hepatic first pass effect. In summary, tramadol administered at 12.5 mg/kg IP provided the longest lasting and most effective analgesia in the rat using the hotplate and tail flick tests. This finding led to additional evaluation of tramadol in a rat surgical model, which is currently under investigation.

## P6 Urinary Catheterization of Male Swine

CM Hite\*

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Urethral catheterization is a common procedure performed on domestic animals to obtain a urine sample, measure urinary output, or relieve urinary bladder pressure from obstruction or during prolonged anesthesia. This procedure, however, has been described as virtually impossible in male swine because of the unique anatomy that presents challenges not found in other species. Alternative techniques have been used, but they have all been invasive, resulting in various degrees of tissue trauma and possible complications. The authors have developed a noninvasive technique of urethral catheterization in male swine using a guide wire and catheter. The authors were successful traversing each anatomic structure and extracting urine on Landrace swine less than 100 kg body weight ( $n = 10$ ) without complications or significant tissue trauma. The technique was performed in less than 15 min after several training sessions.

## P7 Disseminated Mycobacteriosis in a Giant Elephant Shrew (*Rhynchocyon petersi*)

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A 1-y-old male, captive-bred giant elephant shrew (*Rhynchocyon petersi*) presented with lameness of the right hindlimb. Empirical daily therapy with oral meloxicam (0.1 mg/kg) and a 10 d course of cefdinir (10 mg/kg) resulted in minimal improvement. Physical exam and radiographs revealed no remarkable findings. Three weeks later, the fifth digit of the left forelimb exhibited marked swelling. Further, a mass measuring 2 cm × 2 cm × 0.5 cm was palpated at the medial and lateral aspect of the right stifle. Subsequent, repeat radiographs of the affected digit (fifth phalanges) and right stifle (distal femur and proximal tibia) revealed bone lysis and soft tissue swelling. Two weeks after limited success with oral antimycobacterial therapy using rifabutin (20 mg/kg) and azithromycin (15 mg/kg), the shrew became ataxic, lethargic, and anorexic. Shortly after transport to an intensive care unit for supportive care, the shrew was found tachypneic in right lateral recumbency, and was euthanized. Gross necropsy findings revealed an enlarged right stifle joint with multiple coalescing foci of purulent material and pinpoint white foci throughout the lung lobes. Histopathology revealed disseminated mycobacteriosis, which was confirmed by joint culture. Polymerase chain reaction (PCR) demonstrated 100% identity for *Mycobacterium intracellulare*. This case demonstrates that systemic mycobacterial infection is an important differential diagnosis for insectivores presenting with an acute onset of lameness.

## P8 Evaluating the Esthetics of Physical Methods of Euthanasia of Anesthetized Rats

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One of the reasons physical methods of euthanasia are considered conditionally acceptable is the concern that they are esthetically displeasing. This study evaluated the time to euthanasia, time and ease of brain dissection, and esthetics of method of euthanasia for 5 physical methods of euthanasia (decapitation, thoracic percussion, iatrogenic cardiac tamponade, thoracotomy with great vessel incision, and cardiac exsanguination) and 1 nonphysical method of euthanasia (anesthetic overdose) for anesthetized rats. The hypothesis was that all physical methods selected would be equally capable to ensure rapid death for the anesthetized animals. We also hypothesized that methods that caused exsanguination without opening the chest cavity would result in a cleaner and faster brain dissection. Lastly, we hypothesized that methods of euthanasia with little blood would be considered more esthetically pleasing to the human subjects. The first 2 hypotheses were tested by euthanizing 6 rats by each of the described methods and tracking time from induction to death and time from death to complete brain dissection. The last hypothesis was tested by creating a digital film of each euthanasia method and showing it to investigative staff who volunteered to complete our survey. The results of our study showed no significant difference in time of euthanasia or brain dissection among the physical methods of euthanasia.

The research staff reported that they preferred the methods of euthanasia that did not open the thoracic cavity over those that did. This result was statistically significant. The results of this study demonstrate that alternative physical methods of euthanasia, such as iatrogenic cardiac tamponade and thoracic percussion, may be more esthetically pleasing for research personnel and be possible options for decapitation.

## P9 Extreme Susceptibility to Bacterial Infections in a Mouse (*Mus musculus*) Model of Allergen Asthma

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Increased mortality was noted in 2 cohorts of NADPH gp91<sup>phox</sup><sup>-/-</sup> mice used in an allergic-asthma study. NADPH gp91<sup>phox</sup><sup>-/-</sup> mice lack phagocytic superoxide production and have an increased susceptibility to infection. Mice are injected with ovalbumin (OVA) followed by intratracheal allergen challenges to initiate allergen-specific pulmonary disease. Mice are bred in-house at a different location from where experiments take place. The first cohort consisted of 10 female NADPH gp91<sup>phox</sup><sup>-/-</sup> mice. Two weeks after transfer from the breeding facility, and prior to experimental manipulation, 9 of 10 mice were found dead. Pathology findings were consistent with bacterial septicemia; *Stenotrophomonas maltophilia* was cultured. The second cohort of NADPH gp91<sup>phox</sup><sup>-/-</sup> mice consisted of 12 males that were prophylactically administered trimethoprim-sulfamethoxazole for 1 wk prior to manipulation. One control and 1 experimental mouse were found dead on day 21 of the study. A water work-up was performed in the facility that housed the experimental animals. pH was tested on bottles after hydrochloric acid addition as well as on bottles that had been on ventilated cages for 1 to 2 wk; all results were within the accepted range. Bottles and cages were cultured after autoclaving to evaluate sterilization, and the results were negative. Mice from the breeding colony cultured negative after euthanasia. Based on these findings, it was believed the most likely cause for mortality in these mice was the combination of their extreme susceptibility to bacterial infections and the likely stressor of moving between facilities prior to use. Such a stressor likely increased levels of resident bacteria. These bacteria were then spewed back into the water bottle, contributing to the levels of bacteria to which the mice were exposed. To combat this issue, daily water bottle changes were implemented for mice of this strain, after which no mortality was noted. This is an example of the fragility of some genetically engineered mouse strains, and the husbandry modifications they may require for use.

## P10 Large Swine Surgical Model in the Research Setting

DF Funk\*

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Many challenges are encountered in research when you use animals in excess of 350 lb. We have observed an increase in demand for such animals in our research community. Proper handling and knowledge of large animal behavior must be used to ensure the safety of both the personnel and the animal. After testing a number of options, we developed a protocol that promotes safe transport, handling, and anesthesia induction/maintenance of such animals. We have incorporated the use of a modified farrowing pen to transport the animal from their holding pen to the desired location. The fingers on the bottom of the pen were removed and pivoting casters were added to allow the animal to walk to the location encased within the safety of the pen. A 20G butterfly catheter is used to deliver induction drugs intramuscularly. The extension tubing attached to this type of catheter allows you to copy the movement of the animal, decreasing the trauma to the tissue at the injection site. We modified a gallon jug to serve as an induction mask large enough to cover the animal's large snout to deliver oxygen and other needed gases until intubation can be accomplished. We find the use of a Wisconsin size 14 laryngoscope blade, 12 mm (40 cm length) to 16 mm (70 cm length) wire enforced silicone endotracheal tube and a long stylet makes intubation easy while in dorsal recumbency. A hoer hydraulic lift is used to raise and position the animal onto the operating table. With the addition of this equipment, we have been able to successfully safely lift and manipulate these animals without incident or injury to the staff or animal.

## P11 Lymphadenopathy in a Sentinel Mouse

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A single housed female outbred mouse was observed displaying abnormal clinical signs such as a hunched posture, tachypnea, generalized paleness, skin tenting, and trembling. Treatment was initiated with subcutaneous fluids without improvement; euthanasia was ultimately elected. Differential diagnoses included respiratory disease secondary to a viral or bacterial etiologies, anemia, neoplasia, and/or sepsis. Diagnostic tests included hematology, parasitology, serology, peritoneal and anal tapes, and microscopic examination of cecal contents. Severe leukopenia was detected on hematology and *E. coli* and alpha hemolytic streptococcus were cultured from the blood and liver. A necropsy was performed in efforts to find gross or histopathologic changes. Gross pathologic findings included microhepatica and petechiation of all internal organs. Histopathologic findings included purulent pneumonia, enteritis, necrotizing hepatitis, pericarditis, and steatitis. Gram-positive cocci were detected in all organs.

#### **P12 *Mycoplasma ovipneumoniae* Infection in Research Lambs (*Ovis aries*)**

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During 2008, 3- to 8-mo-old male and female Dorset-Finn lambs ( $n = 12$ ) were acquired for cardiovascular and respiratory studies. Lambs were treated prophylactically on arrival for "shipping fever" with oxytetracycline every other day for 1 to 3 treatments, based on the supplier's recommendation. Despite prophylaxis, all lambs developed clinical signs of respiratory disease, including purulent nasal discharge, cough, and dyspnea, that were unresponsive to treatment with cephalosporin and lincosamide antibiotics. At necropsy there was gross pulmonary consolidation with mucus casts as well as microscopic moderate to severe lymphocytic and proliferative bronchitis/bronchiolitis with bronchiectasis (cuffing pneumonia) suggestive of *Mycoplasma ovipneumoniae*. Paired serology (indirect hemagglutination test, Washington Animal Disease Diagnostic Laboratory) on 10 lambs showed high antibody levels ( $> 1:2560$ ) for *M. ovipneumoniae*. The receiving protocol was changed to include florfenicol (20 mg/kg IM every other day for 3 treatments). Lambs treated with this regimen had no detectable decrease in antibody levels 6 wk after treatment, compared with pretreatment serology. The receiving protocol was amended again to include a second course of florfenicol 10 d after the end of the initial treatment. One lamb treated with 2 courses of florfenicol had a 4-fold decrease in titer with mild histologic lesions in the lungs. In summary, chronic atypical bronchopneumonia in lambs less than 1 y old have complicated ongoing cardiovascular and respiratory research studies. Treatment with florfenicol has reduced clinical signs of bronchopneumonia but has not eliminated correlative histopathologic findings in the lungs.

#### **P13 Clinical Signs of the Acute and Prolonged Radiation-Induced Gastrointestinal Syndromes in Rhesus Macaques**

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The gastrointestinal (GI) system is a significant concern for those exposed in radiation accidents, nuclear terrorist events, and cancer therapies. The GI subsyndrome of the acute radiation syndrome (ARS) induces severe dehydration and diarrhea due to the ablation of intestinal crypts and villi resulting in lethality within 6 to 10 d. Currently, there are no Food and Drug Administration (FDA)-approved treatments for the radiation-induced GI syndrome. The long-term clinical consequences of otherwise lethal irradiation are only beginning to be understood. While developing an animal model for the efficacy testing of GI ARS drugs/biologics under the FDA Animal Rule criteria, we observed 2 stages of the GI syndrome, acute and prolonged. Rhesus macaques received acute total body irradiation (TBI) with or without 5% bone marrow (BM)-sparing (TBI/BM5) at doses from 10.0 to 14.0 Gy to induce acute and prolonged GI syndromes. The TBI/BM5 model allowed recovery from the otherwise lethal hematopoietic syndrome. Post irradiation animals were provided full supportive care including hydration fluids, antibiotics, antidiarrheals, supplemental

nutrition, analgesics, and antiulceratives per daily assessment of clinical signs. GI and hematopoietic parameters were assessed until the primary endpoint (survival to 180 d after treatment) was realized. The presentation of factors contributing to severe dehydration and morbidity were dose- and time-dependent upon the stage of the GI syndrome. Severe dehydration and diarrhea were the hallmarks of the acute stage. In the prolonged stage these conditions were less severe, yet persistent. However, animals experienced edema, and continued weight loss was a predominant feature of the prolonged syndrome which ultimately resulted in IACUC-mandated euthanasia. In summary, despite the use of supportive care, which improved survival during the acute stage, overall mortality was inevitable during the prolonged stage of the GI syndrome.

#### **P14 An Effective Venipuncture Technique and Normal Serum Chemistry Parameters of the Captive Fat-Tailed Jird (*Pachyuromys duprasi*)**

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Thirty-nine captively reared fat-tailed jirds (*Pachyuromys duprasi*) were enrolled in a minimally invasive study to determine an effective venipuncture technique and establish normal serum chemistry parameters. A jugular venipuncture technique using chemical restraint (ketamine 30 mg/kg, xylazine 6 mg/kg, and acepromazine 1 mg/kg administered intraperitoneally) was found to consistently yield a sufficient blood volume. Serum chemistry parameters were established for glucose, total protein, albumin, alkaline phosphatase, alanine transferase, total bilirubin, amylase, blood urea nitrogen, creatinine, calcium, phosphorous, sodium, and potassium. Amylase and glucose levels were found to be significantly ( $P < 0.05$ ) different between males and females.

#### **P15 A New Method of Repeated Blood Collection from Dorsal Metatarsal Vein in Mice**

H Azabu<sup>1</sup>

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In experiments using small animals like mice, blood collection from the jugular vein, tail vein, or orbital sinus veins have been used. However, these methods may not be appropriate for frequent and small blood sampling, such as in pharmacokinetic studies, because of requirements for higher skills of technicians and significant damage to the small animals. With this in mind, we have successfully established a new, less invasive, simple, and convenient blood sampling method from dorsal metatarsal veins using hematocrit capillary tubes and needles under consciousness. Using this method, excessive amount of blood sampling cannot be removed and adjustment of the amount is possible. In addition, we believe that the animals can recover quickly and the stress to the animals should be smaller, due to their short restriction, and that mis-needlestick can rarely happen owing to their visible veins. Introduction of this method has brought us to more efficient performance of the experiment at higher quality than before.

#### **P16 Vomiting and Respiratory Signs in Four Parkin Gene Knockout Mice**

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Four adult agouti mice from a breeding colony were identified as health cases by the husbandry staff due to vomiting. These mice were housed 2 per cage, ranged in age from 2 to 6-mo-old, and were 129S4 parkin gene knockout mice, and therefore models for Parkinson disease. Evaluation by a veterinarian in the vivarium revealed hunched postures, ruffled coats, mildly labored breathing, and clear ocular discharge. The laboratory opted for euthanasia and agreed to transfer the animals to the necropsy room. On physical exam the first male mouse was quiet but responsive, had a decreased activity level, an occasional hunched posture, labored breathing, increased respiratory rate, and a body condition score of 3 out of 5. A complete blood count revealed a moderate neutrophilia, mild polycythemia, and mild thrombocytopenia. There were no abnormal values on a chemistry 21 panel. A physical exam of the other 3 mice, 2 males and 1 female, revealed that they were bright, alert, and active, and had a body condition score of 3 out of 5. Changes on the complete blood



counts included a mild polycythemia and mild thrombocytopenia for all 3 mice. In addition, the third male had a mild monocytosis and the female had a mild neutrophilia. Necropsy revealed dilation of the esophagus from the esophageal hiatus of the diaphragm to the thoracic inlet in all 4 mice. The final diagnosis was megaesophagus. To our knowledge, this is the first reported case of megaesophagus in a parkin gene knockout mouse.

#### **P17 *Mycobacterium liflandii* Outbreak in a Research Colony of *Xenopus tropicalis***

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A research colony of *Xenopus tropicalis* housed in individual static tanks within a room presented with skin lesions. Clinical signs were first noted in a single tank, but eventually appeared in frogs housed in all tanks within the room over a period of 3 mo. The skin lesions were multifocal, ranging from small tan-red nodules to flat white discoloration or red ulcerated lesions. Copious acid-fast positive organisms were noted on staining of skin impression smears by Zeihl-Neelson stain. Frogs were systematically depopulated and necropsied from each affected tank. Additional consistent gross findings were enlarged tan-yellow spleen and multiple yellow-white miliary to nodular foci on the liver. Histopathologic findings included necrotizing granulomatous dermatitis, splenitis, and hepatitis with numerous intralesional acid-fast bacilli consistent with systemic mycobacteriosis. Representative samples of the affected organs were submitted for bacterial isolation and characterization. Mycobacteria grown on Lowenstein-Jensen (LJ) media were genetically characterized by PCR amplification of the enoyl reductase (ER) domain present on the mycolactone plasmid found in *M. ulcerans* and *M. liflandii* and by identifying specific variable numbers of tandem repeats (VNTR) at locus MIRU1, locus 6, ST1, and locus 19. PCR results confirmed infection with *M. liflandii*. Mycolactone E was characterized by extracting ethanol soluble lipids (ESLs) from bacterial cells isolated from diseased frog tissue and comparing against an *M. liflandii* control on thin layer chromatography. *M. liflandii* in recent years has had a devastating impact on research frog colonies throughout the United States since its first identification in an outbreak of mycobacteriosis in a colony of *X. tropicalis* in 2001. Further studies are needed to characterize the pathogenesis of *M. liflandii*, as well as development of optimal management and preventive measures to minimize the impact of this disease.

#### **P18 Botryomycosis with Eosinophilic Ym1-positive Crystals in a Transgenic Mouse**

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A 3-wk-old, male Z/EG transgenic mouse presented with a swelling in the left periorbital tissue. The mouse was carrying the lacZ gene that is crossed with other genetically altered mice to determine the tissue specific expression of a Cre-recombinase transgene. Gross findings included a 1.4 cm × 1.4 cm × 0.5 cm multilobulated tan, soft mass dissected at necropsy from subcutaneous and periocular soft tissue ventral to the left eyeball. Histologic examination of the mass showed focal, severe, pyogranulomatous panniculitis, cellulitis, myositis, and Harderian gland adenitis with numerous bacterial colonies (primarily gram-positive cocci), and numerous eosinophilic, acicular crystals. The findings were consistent with botryomycosis, a term for granulomatous inflammation, typically caused by *Staphylococcus aureus*. The feature of this case was the formation of eosinophilic acicular crystals, which are not usually seen with botryomycosis. Eosinophilic crystals in mice have been documented in the glandular stomach, lower pulmonary tract, bile duct, and gall bladder. Immunohistochemistry staining indicated that the eosinophilic crystals were positive for Ym1 protein. The function of this protein remains unclear.

#### **P19 Effective and Safe Anesthesia for Yorkshire and Yucatan Swine with and without Cardiovascular Injury and Intervention**

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We investigated an injectable anesthetic protocol that provides sedation sufficient for catheterization, intubation, and transport while minimizing cardiovascular changes in Yorkshire and Yucatan pigs with and without cardiovascular injury and intervention (CI). Phase 1 examined the safety and efficacy of acepromazine/ketamine, diazepam/ketamine, midazolam/ketamine, and medetomidine/ketamine in 5 healthy Yorkshire pigs. For each drug combination, we obtained multiple measurements of heart rate, blood pressure, respiratory rate, temperature, sedation score, ability to catheterize and intubate, and recovery score. Phase 2 evaluated and refined the dose of the best Phase 1 anesthetic combination (midazolam/ketamine) in healthy and CI Yorkshire pigs (*n* = 53 trials). Phase 3 mirrored Phase 2 but tested midazolam/ketamine in healthy and CI Yucatan pigs (*n* = 34 trials). Midazolam (0.5 mg/kg) and ketamine (25 to 27 mg/kg) was the most effective anesthetic in healthy Yorkshire pigs, but this dose was less effective in healthy Yucatan pigs. This dose was also less effective in CI Yorkshire and Yucatan pigs. Midazolam/ketamine resulted in tachycardia and apnea more frequently than in healthy pigs. This combination also caused vomiting in 1 CI Yucatan pig. Overall, midazolam/ketamine provided safe and effective sedation for catheterization and intubation of both healthy and CI pigs. This study suggests that Yucatan pigs may require a higher dose midazolam/ketamine to achieve the same level of sedation as Yorkshire pigs. While the anesthetic complication rate increased in CI pigs, our results indicate that midazolam/ketamine can be safely used for sedation of both pig breeds with and without CI.

#### **P20 Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Random-Sourced Dogs Used for Teaching Purposes**

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This report documents the occurrence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in dogs used for teaching and outlines the policy instituted for future MRSA management. Between November 2008 and January 2009, MRSA infections were confirmed in 4 random sourced teaching dogs at Purdue University School of Veterinary Medicine. The organisms were isolated from 3 hound dogs, between the ages of 2 and 3 y, and one 6-y-old male chocolate Labrador retriever. Repeated complete physical examinations were performed on these dogs by veterinary students and veterinary technicians, and no previous lesions were seen that suggested a developing Staphylococcal dermatitis. The clinical signs noted in the hounds included alopecia, redness, and flaking over the affected areas of body while the Labrador retriever developed tiny pustules under its chin. The differential diagnoses for the skin lesions included dermatophytosis, contact dermatitis, mite infestation, and umbilical herniation. The lesions were treated with topical antibiotic/steroid ointments. When the lesions failed to resolve after treatment, samples including skin biopsies and skin, nasal, and rectal swabs were taken for culture and cytologic evaluation. All animals had MRSA infection confirmed by bacterial isolation and polymerase chain reaction (PCR). The hounds were euthanized for other reasons, while the Labrador retriever was treated with chloramphenicol at a dose of 60 mg/kg TID for 4 wk. The Labrador retriever was retested and confirmed negative for MRSA by culture and PCR 3 wk after treatment. The occurrence of these cases prompted the animal husbandry and care administrative staff to design a new policy that applies to all species of animals in the facility. The new policy provides guidelines for suspecting and testing for MRSA infection, as well as a protocol to follow when MRSA infection is confirmed.

#### **P21 The Use of Environmental PCR Swabs to Identify Mouse Parvovirus (MPV): Determining the Effectiveness of Room Decontamination Procedures Using PCR**

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In 2007/2008, a comparative study was conducted to evaluate the use of environmental PCR swab testing versus the use of sentinel animals to determine the effectiveness of room decontamination procedures after MPV outbreaks. Past practices for MPV decontamination involved room depopulation, sanitization, and decontamination procedures for 3 cycles. Sentinels would be placed in the room for 6 wk, and then tested for MPV. If serology/tissue test results were negative, the room would be repopulated.

On several occasions, after repopulation with study animals, sentinels would seroconvert and become MPV positive (approximately 5 of 20 animals per room). Currently, MPV positive rooms are depopulated, sanitized, and decontaminated, followed by testing of environmental PCR swabs. In this study, most rooms were negative after decontamination, but in some cases, approximately 2 of 6 swab samples per room would test positive for MPV after the first round of decontamination. The room would again be sanitized and decontaminated repeatedly until all swab samples were negative. Since we have initiated this process, 100% of rooms screened by PCR swabs have not tested positive for MPV after repopulation with study animals. This new PCR methodology uses 6 sterile swabs per room to sample environmental surfaces (floors, vents) and equipment (hoods, computers) to detect the presence of MPV in depopulated rooms. Based on our experience, these locations/objects were identified to be the best sources to test for MPV DNA to determine effectiveness of decontamination procedures. This has allowed the elimination of sentinel animals and has decreased "down time" from a process of 8 to 12 wk, to 4 to 5 wk, which includes cleaning/decontamination, swab collection, lab analysis, and the interpretation of results. The use of environmental PCR swab testing has increased the incidence for detecting MPV prior to repopulating a room with study animals and this has decreased the time to turnover rooms for study use. This new procedure has allowed the incorporation of the 3 Rs and increased our confidence in the use of PCR technology compared to animal use. It has allowed us to eliminate the use of animals, refine technology by using PCR, and has reduced the loss of productivity and delayed studies.

### **P22 Containment of an Epizootic Diarrhea of Infant Mice Outbreak in a Modified Rodent Barrier**

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Rotavirus A (RV-A) or epizootic diarrhea of infant mice (EDIM) has been reported to be a highly contagious pathogen. However, a recent outbreak at our institution indicates that personnel and fomites may not be significant instruments of dissemination. In February 2009, an outbreak of EDIM was identified in 1 room (550F) of a modified rodent barrier via sentinel testing. In 550F, immunocompetent mice were tested via serology and immunocompromised NOD scid gamma (NSG) mice were tested via fecal PCR. Results revealed that the majority of the NSG mice were viremic, and although some immunocompetent mice were seropositive for antibodies, none were viremic. Moreover, cages with mice that were weaned from seropositive immunocompetent cages were found to be seronegative. Once the infection was confirmed, monthly testing was instituted in the additional 10 modified barrier rooms and 14 mouse rooms found on different floors of the same facility. Six months following the initial identification of the outbreak, the remaining rooms have continued to be negative for evidence of exposure to the disease. The presence of severely immunocompromised animals and intensive breeding created conditions conducive to propagating the rotavirus within 550F. Upon analysis of the traffic patterns of personnel and equipment prior to the discovery of the outbreak, it was determined that an inhalant anesthesia machine and a carbon dioxide euthanasia chamber had moved freely between all rooms in the modified barrier. In addition, animal care staff had served rooms prior to and following the affected room. Although gloves were changed between rooms, gowns and booties were not. As cross-contamination could have easily occurred with either equipment or husbandry staff, the lack of evidence of such indicates that the transfer of the virus was more likely from close animal contact than from personnel or fomites.

### **P23 Cardiopuncture as Method of Blood Collection Causes Physiologic Complications in Japanese Quail**

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We evaluated physiologic responses in Japanese quail following blood sampling from 3 sites: brachial (wing), jugular, and cardiac. Twenty-four Japanese quail (*Coturnix japonica*) layers were randomly divided into 3 groups of 8 birds each. To minimize the effect of age and physiologic status, birds of similar age and body weights, and having hard-shelled eggs in the uterus at the time of blood sampling were used. The birds were housed in wire cages under 14:10 h dark/light period. Feed and water were with-

held for 4 h prior to sampling. Birds were deeply sedated and immobilized with Isoflurane. Blood (approximately 0.10 mL) was collected in capillary tubing containing EDTA from the brachial vein and the jugular vein (using a lancet) and from the heart using a tuberculin syringe fitted with 25G needle (1.5 in.) entering through clavicles. Blood sampling from brachial and jugular was simple; however, blood leakage under the skin occurred in approximately 10% of the birds in both groups. Two birds from both the brachial and jugular venipuncture groups skipped laying for 1 to 2 d with no significant loss in body weights. Whereas, in the cardiac group, 2 birds succumbed on the spot and the remaining 6 birds lost significant weight (120.5 g versus 130.2 g;  $P < 0.05$ ) and stopped laying eggs for the next 5 to 7 d. During that period, the birds had elevated Hb (11.6 g versus 10.8 g;  $P > 0.05$ ) as well as PCV (40.1 versus 36.5;  $P < 0.05$ ) values. As the birds in the cardiac group resumed egg production, both Hb and PCV returned to close to pretreatment values; however, they did not regain pretreatment body weight. At this time, all the birds in the cardiac group were euthanized with CO<sub>2</sub>. Two of the euthanized birds exhibited hemopericardium, which upon histologic examination, exhibited collagenous scar tissues and ventricular hemorrhages along the path of needle entry. In summary, jugular and brachial venipuncture are safer, with only low grade complications, whereas cardiac puncture procedure is traumatic and possibly fatal, with deleterious effect on ongoing experimentation.

### **P24 Balanoposthitis in Glycosuric Zucker Fatty (fa/fa) Rats**

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Inflammation and infection of the glans penis and prepuce (balanoposthitis) were noted in an experimentally naïve group (10) of diabetic male Zucker Fatty rats (fa/fa). Rats presented at 4.5 to 5-mo-old with evidence of glycosuria and in some cases dysuria. Preputial tissues were swollen and erythematous. A thick, suppurative discharge was present, often forming plugs/nodules and adhering to the urethral meatus, glans penis, and preputial skin. Nonglycosuric cohorts were not affected. Cytology of the exudate revealed a large number of neutrophils, various bacteria (primarily coccil), fungal hyphae, and yeast. Swab cultures from affected animals revealed the presence of *Escherichia coli*, *Enterococcus* spp., beta hemolytic streptococcus, and coagulase positive *Staphylococcus* spp. as well as *Candida parapsilosis*. *E. coli* and *C. parapsilosis* were identified from all samples submitted ( $n = 4$ ), while the remaining microbes varied. Swab cultures from nonaffected animals identified *E. coli* and *Enterococcus* spp. Experimental parameters did not allow for systemic or topical treatment with antibiotics or antifungals. Daily cleansing of the affected areas and application of povidine-iodine were instituted. Marked reduction in the amount of exudates and inflammation were noted at the end of 7 d. *C. parapsilosis* is an increasingly emerging opportunistic, nonalbicans pathogen that has been reported with increased frequency in the human population. Veterinary cases of mastitis, cystitis, endocarditis, dermatitis, and abortion across various species have been reported. We believe this is the first case of *Candida parapsilosis* balanoposthitis reported in rats.

### **P25 The Effects of Postoperative Analgesia on Alcohol Consumption in Mice**

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Withholding postoperative analgesia is routinely requested by investigators in behavioral studies with a surgical component due to possible confounding effects for research outcomes. Buprenorphine, a partial mu opioid receptor agonist, has the benefit of a prolonged duration of action, which is useful from an animal welfare perspective; however, this could possibly interfere with behavior research outcomes. Nonsteroidal antiinflammatory drugs (NSAIDs) can offer an alternative to the side effects of opioids. Injectable ketorolac, an NSAID commonly used for analgesia in veterinary patients, is a nonspecific cyclooxygenase (COX) inhibitor. However, COX inhibitors have been shown to have a variety of effects on ethanol drinking behavior within 24 h after administration. In this study, we use a rodent model (C57BL/6 mice who readily consume alcohol when given a choice between 10% ethanol solution and water) to determine whether a single postoperative dose of buprenorphine

or ketorolac following surgery produces behavioral or biochemical effects at 7 to 18 d post administration. Our findings illustrate that there is no statistically significant effect on ethanol consumption (a common behavioral study template) by providing a single dose of analgesia postoperatively. Specifically, the average ethanol intake after 1 injection of buprenorphine or ketorolac or saline control in respective groups of 10 subjects did not deviate significantly from the values of 0.88 to 0.96 on day 1 to 0.74 to 1.34 on day 7, and on day 15, the standard deviation was 0.63 to 1.35. The significance of this finding is that there is support for providing postoperative analgesia for those animals undergoing behavioral studies without a negative impact upon that study. As a result, this can lead to refinement in future behavioral studies.

#### **P26 Anesthetic Protocol for an Extended Duration Electrophysiology Study in Cynomolgus Macaques**

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Electrophysiologic mapping of motor outputs evoked by microstimulation in the brain was performed twice for 2 cynomolgus macaques (*Macaca fascicularis*). The duration of anesthesia ranged from 12 to 20 h. In order to correlate microstimulation with muscle movement, an anesthetic plan to produce unconsciousness without inhibiting muscle movement was developed. Volatile anesthetics, such as isoflurane, could not be used because they act primarily in the CNS to limit initiation of action potentials and produce immobility; therefore, correlation of microstimulation with muscle movement would be impossible. Ketamine was the ideal selection since it is a dissociative anesthetic, producing unconsciousness but not inhibiting muscle stimulation. Since each animal exhibited a different sensitivity to ketamine, it was necessary to titrate a continuous rate infusion on an individual basis. Additionally, 5 mcg/kg of dexmedetomidine, an alpha-2 agonist, was administered every 2 h to compensate for the poor muscle relaxant and analgesic effects of ketamine, stabilize hemodynamic responses, and reduce the amount of ketamine required to reach an appropriate plane of anesthesia. Mannitol was provided every 6 to 8 h to reduce intracerebral pressure and prevent edema. Fluid balance was carefully monitored; urine output was measured and fluids were increased to compensate for mannitol administration. Total fluid rate was between 5 to 10 mL/h, and was supplied via 2 formulations: one as the ketamine infusion and the other as a 2.5 or 5% dextrose solution. Lactated ringers solution (LRS) was initially selected for both formulations as a means to preemptively counteract anticipated acidosis. In the cases of metabolic alkalosis, LRS was replaced with 0.9% saline. Venous blood gases were run every 2 to 4 h, and electrolyte imbalances corrected as needed. Both procedures for each patient were successful; the anesthetic plan provided sedation and analgesia as well as an appropriate plane of anesthesia for electrophysiologic mapping. This plan has potential application for any surgical procedure requiring dissociative anesthesia without total immobility.

#### **P27 Technological Study on Sperm Cryopreservation in C57BL/6J Mice and Other Genetically Manipulated Mice**

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Male C57BL/6J mice and other genetically manipulated mice (also with C57BL/6J background) were used to compare the effects of cryopreservants, sperm capacitation time and fluid, and IVF medium on sperm cryopreservation. Mice were euthanized by cervical vertebrae dislocation, and the caudae epididymides were removed. The freezing and thawing of sperm were based on Nakagata method, which uses 2 thawing methods when R18S3 is used as a cryopreservant: 37 °C water bath for 15 min, and 60 °C water bath for 6 s followed by 37 °C water bath for 10 min. For this experiment, we used the latter thawing method, as there were no significant differences in IVF cleavage rates ( $P > 0.05$ ). Sperm capacitation times were 30, 50, and 80 min, respectively; the best time was 50 min. Addition of 1.0 IU/mL FSH or 0.1 µg/mL E2 into 100 µL HTF solution as the sperm capacitation medium resulted in 24.1% and 27.3% of cleavage rates for cryopreserved sperm IVF and no difference for the control (25%,  $P > 0.05$ ). Based on former work, we used R18S3 alone and with 5%, 10%, 15%, 20%, 25%, and 30% yolk supernatant as the cryopreservants. The results showed that when the cryopreservant was R18S3 + 20% yolk supernatant, the IVF rate was highest, about 40%, compared with 20% when

the cryopreservant was R18S3 alone. The addition of different concentrations of glycerol or acetamide would decrease the cleavage rates of IVF embryos. The addition of yolk plus glycerol yielded worse freezing results than that of yolk addition alone, but better results than using glycerol alone. When adding 5 µmol/L adrenaline, 75 µmol/L hypotaurine, 5 IU/mL heparin, or 7.5 mmol/L taurine into 100 µL HTF solution as sperm capacitation factors, only the 5 IU/mL heparin group could be increased slightly for the R18S3 group. Addition of 45 µmol/L adrenaline resulted in the highest IVF rate for the R18S3 + 20% yolk. All the 2-cell IVF embryos developed into blastocyst in KSOM medium, with a birth rate of approximately 30% for these embryos. In conclusion, R18S3 + 20% egg yolk supernatant as a new cryopreservant was more effective than R18S3 alone.

#### **P28 Torticollis in *Mycobacterium tuberculosis*-Infected Mice**

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Tuberculosis (TB) remains 1 of the most prevalent worldwide infectious diseases causing morbidity and mortality in human beings. Mouse models have helped advance vaccine and drug development for treating this devastating disease. Between day 52 and 5 mo after infection, 10 of 170 mice infected by intravenous inoculation of  $5 \times 10^6$  CFU of *Mycobacterium tuberculosis* strain Erdman (ATCC 35801) developed torticollis. This included mice in treatment groups that received combination antibiotic therapy of rifampin/pyrazinamide or moxifloxacin/rifampin/pyrazinamide. Torticollis did not develop in mice receiving isoniazid/rifampin/pyrazinamide therapy, nor was it present in the cohort of aerogenically infected mice. The mice were euthanized and complete necropsy evaluation was performed on 4 mice. Gross necropsy evaluation revealed typical TB lesions in lungs of infected mice. Histologic evaluation of tissues also revealed a granulomatous otitis media with intralosomal acid fast bacilli consistent with *Mycobacterium tuberculosis*. These cases represent an unusual finding specific to the intravenous mouse model and may inform investigators about drug penetration issues.

#### **P29 Peripherally Inserted Central Catheters (PICC) for Multiple Blood Collections**

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In current research projects at our institution, there is a strong demand for repeatable time-dependant blood sampling in rhesus (*Macaca mulatta*) or cynomolgus (*Macaca fascicularis*) macaques. Previously, repeated blood draws were done under anesthesia using venipuncture of the femoral vein. This technique, albeit successful, had serious disadvantages in that there was often bruising of the femoral triangle, concern over missing critical timepoints, and the possibility of creating arterial-venous fistulas. As a result, we developed a protocol to temporarily use a peripheral intravenous central catheter (PICC) for short-term (< 2 h) repeated venipuncture (> 5 in a 1 h period). The animals are sedated with ketamine, intubated, and anesthetized with isoflurane for the duration of the procedure. At the time of catheter removal, the placement site is observed for any abnormalities and then observed twice daily thereafter. In addition, we adapted this procedure to allow for infusion of possible therapies into the central venous system. This technique was used in multiple studies and resulted in less trauma due to the need for only 1 catheter placement. PICC is a commonly used technique in human medicine for a variety of purposes, including infusion of chemotherapeutic agents and blood draws. Concerns of using PICC include thrombus formation, septic emboli, and arrhythmias due to poorly placed catheters. We have not observed any of these concerns possibly due to the short use of the catheters, strict aseptic guidelines, and proper maintenance of the catheter in situ. Overall, the adoption of the use of PICC for multiple blood draw protocols has been successful at our institution and a refinement from repeated venipuncture of the femoral vein.

#### **P30 Predictive Models of Growth and Male Sexual Maturity in the Cynomolgus Monkey (*Macaca fascicularis*)**

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The cynomolgus monkey (*Macaca fascicularis*) is an important animal model in basic research and safety testing. Being able to predict the growth and sexual development of this animal refines our ability to assess drug effect versus normal biologic variability, select a sexually mature animal for reproductive toxicity evaluations, and provide more comprehensive veterinary care. To that end, we provide 2 statistical models for growth and male sexual development in the cynomolgus monkey based on a retrospective analysis of data from our medical records and nonclinical safety study reports. In the first model, body weight measurements were summarized by 3 curves representing the median (M), the coefficient of variation (S) and the skewness (L) as they change with age. Statistical smoothing techniques and nonlinear regressions were then applied to the data based on the model of Centile =  $M(1+LSZ)^{1/L}$  to establish the L, M, and S curves for each age group, centile curves, and finally a growth chart for the cynomolgus monkey. In the second model, variability in sexual maturity is addressed and an accurate prediction model of sexual maturity, based on body weight and age with 95% confidence interval and ROC curves, was established. The algorithm is implemented by fitting the histologic assessment of testicular maturity against body weight and age using a binary logistic regression method. About 90% sensitivity was achieved with this mathematical model.

### P31 Clinical Syndromes in Axenic Swiss Webster Mice

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Swiss Webster mice with complex flora are commonly used in colony management for their hybrid vigor, characterized by large litters, robust immune response, and relative lack of spontaneous disease. Swiss Webster mice are the only commercially available axenic outbred mice and serve a critical role as recipient females in embryo rederivation of other mouse strains into a germfree status. After more than 5 y of managing a small germfree colony, we have observed spontaneous clinical syndromes uncharacteristic of Swiss Webster mice with complex flora. As axenic Swiss mice age, pigmentary gallstones, idiopathic in cause, develop in more than 50% of the colony maintained on a standard breeder chow. Necropsy has revealed dilated gallbladders with minimal inflammation. The cause of stone formation may be related to poor gallbladder motility as is universally observed in the dilated cecum of germfree mice. Ulcerative dermatitis in C57BL/6 mice is a common syndrome characterized by autoimmune-like vasculitis, opportunistic infections, and is impacted by dietary factors. Germfree Swiss mice develop ulcerative dermatitis lesions with similar histopathologic features in the absence of known contributory factors such as autoimmune disease, bacteria, or ectoparasites. One case of rectal prolapse was observed; this is commonly encountered in immune dysregulated mice, often in response to intestinal flora such as *Helicobacter*. Necropsy of this male Swiss mouse revealed the cause to be related to accessory sex gland abnormalities that presumably caused this mouse to strain during urination or defecation. These clinical syndromes in axenic Swiss mice are valuable for their potential contribution to establishing their etiology and in the case of pigmentary stones, are a promising animal model of human disease.

### P32 Research Macaques and Pinworms: A Sticky Business

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Macaques used in biomedical research can harbor a number of zoonotic diseases and likewise are susceptible to a spectrum of human pathogens. During quarterly roundup exams of around 100 animals, 2 clinically healthy rhesus males were diagnosed as having nematodes compatible with *Enterobius vermicularis*, the human pinworm. Adult worms were visible in the fecal sample collected from 1 animal while, in the other, pinworm eggs were evident using a standard fecal flotation technique. The infested animals were housed in different rooms joined by a common vestibule, and all 23 macaques in the 2 rooms were treated twice with injectable ivermectin. Multiple fecal exams (direct smear and fecal flotation techniques) following treatment were negative for endoparasites, as were similar fecal exams of the entire colony at the next roundup. In various animal species, and recently in owl monkeys, perianal tape tests have been shown to be much more sensitive than fecal flotation techniques for diagnosing pinworms;

perianal samples are also collected from children for pinworm testing. Thus collecting perianal tapes was added to the existing regimen for endoparasite testing at a subsequent colony roundup. Two paired females and 1 singly housed male, housed in rooms other than those with pinworms earlier, were found perianal tape-test positive for pinworms; all other fecal tests from the colony were pinworm negative. These results highlight the value of perianal tape testing in nonhuman primate colonies where pinworms are a potential concern. The source of the infestation is speculative at present, and perianal tape testing has been added to the procedures carried out on newly arrived animals in quarantine. Human pinworms have been characterized especially in captive simian primates, usually with no clinical signs. Nevertheless, possible transmission between animals and facility/research staff mandates continued surveillance and eradication.

### P33 Use of p63, a Myoepithelial Cell Marker, in Determining the Invasiveness of Spontaneously Occurring Mammary Neoplasia in a Rhesus Macaque (*Macaca mulatta*)

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Spontaneous mammary neoplasms occur infrequently in nonhuman primates. Here we describe a case of mammary gland ductal carcinoma in an aged rhesus macaque. Tissue from an antemortem biopsy of the mass was stained using routine hematoxylin and eosin methods. Tumor invasiveness was further characterized by p63 immunohistochemistry. A p53 homologue, p63 strongly and specifically stains nuclei of myoepithelial cells in human and canine breast tissue. Since p63 has an affinity for the nucleus of myoepithelial cells, it is readily visible, whereas other markers, such as alpha smooth muscle actin and vimentin, are cytoplasmic and may not be as easily discernable from surrounding cells. P63 staining of mammary tissue from the monkey demonstrated foci of neoplastic cells that breached the myoepithelial cell layer surrounding ducts, confirming local invasion of the tumor. To the authors' knowledge, this is the first documented use of p63 as an effective means of staging invasive mammary tumors in nonhuman primates. As nonhuman primates are important animal models for human diseases, including neoplasia, this method may prove useful for both diagnostic and research purposes.

### P34 Rodent Dissection to Pathologist's Slide Evaluation: Achieving Optimal Results

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We will outline the important steps necessary in order to achieve optimal histologic data for a pathologist's slide evaluation. Slide quality is a combined result of proper dissection techniques, fixation, trimming, processing, and embedding. In the pathology department at our facility, guidelines have been set up by the pathologists and technicians in order to standardize these procedures so as to avoid technical discrepancies. The process begins at dissection; if tissues are not dissected with care and fixed properly, the end result of quality slides will not be accomplished. A necropsy dissection guide has been created for both mice and rats, demonstrating the best possible way to dissect tissues for optimal fixation. Inadequate fixation could result in autolysis of the tissue, poor staining, and may affect the ability to detect lesions on the final slide. For example, tissues such as the lung are infused with 10% formalin until the margins of the lobes are filled so that penetration of the exterior cell layers as well as interior cell layers is achieved. Tissue trimming and embedding guidelines have also been created for mouse and rat tissues to correlate with the dissection guidelines. Certain technical procedures, such as specific lobes or areas to be trimmed, the number of sections to be trimmed, the thickness of the sections, and the direction of trimming (for example, cross section, longitudinal), have been standardized for each tissue. By following these guidelines, improved consistency and quality has been noticed by the pathologists. Both guides have also proved to be a useful tool for training new employees as well as providing a reference for current staff.

### P35 Electrophysiologic Cardiac Evaluation by Magnetocardiography in Nonhuman Primates

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Magnetocardiography (MCG) is a new modality that uses superconducting quantum interference devices to detect and visualize weak cardiac electrical activation. The advantages of MCG are high sensitivity to small signals, lack of influence by body tissues, and noninvasive recording of signals. Cardiovascular disease (CVD) is the leading cause of human morbidity and mortality. Therefore, we require nonhuman primate models of CVD that reliably mimic human diseases so that causative mechanisms can be investigated and novel drugs, diagnostic procedures, and therapies can be developed. The present study highlights the development of MCG in nonhuman primates and its potential for clinical practice. We designed an MCG system for nonhuman primates using 64-channel gradiometers in a shielded room with an animal monitoring system. We obtained MCG data from 95 cynomolgus monkeys (51 females and 44 males) bred at the Tsukuba Primate Research Center. The MCG data were generated as images of averaged waveforms and magnetic field maps. We collected normal images of averaged waveforms and magnetic field maps equivalent to those of humans and established a means of electrophysiologic cardiac evaluation in nonhuman primates. The averaged waveforms determined normal values of MCG parameters, such as PQ interval, QRS duration, QT interval, and QTc, in cynomolgus monkeys. This MCG system and normal values will support studies of nonhuman primate models for CVD. Noninvasive and highly sensitive MCG techniques are suitable for animal experimentation, particularly in nonhuman primates. We also extracted MCG images reflecting symptoms of cardiomyopathy, arrhythmias, such as bundle branch block, long QT syndrome, WPW syndrome, and Brugada syndrome. Establishing these models of CVD in nonhuman primates might be particularly useful for understanding the biologic aspects of cardiology. In summary, electrophysiologic cardiac evaluation systems based on MCG that can evaluate nonhuman primate CVD will lead to novel models of safety assessment.

### **P36 Lessons Learned in Regulating Prothrombin Time in a Canine Model Using Warfarin Sodium as an Anticoagulant**

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As part of an approved protocol to evaluate safety, 3 groups of canines were implanted with medical devices. One of the 3 study groups required warfarin to be administered daily with the goal of obtaining a prothrombin time (PT) of 2 to 3 times baseline throughout the study. Initially, canines were administered warfarin at a dose of 0.2 mg/kg PO q 24 h the day after surgery with supplemental heparin boluses of 100 units/kg SC twice daily for the first 2 d. In addition to warfarin, all canines received a nonsteroidal antiinflammatory drug (NSAID) the day before surgery through day 4 postoperatively as 1 element of our multimodal analgesia. The potentiated sulfonamide antibiotic was used postoperatively. Published information is lacking to determine how to adjust the dose of warfarin in canines, only that adjustments should be made to achieve specific PTs. Within 1 wk of implant, 3 of the 4 dogs experienced acute bleeding episodes severe enough to require vitamin K administration. One dog required a blood transfusion. Further investigation revealed warfarin interacts with NSAIDs and potentiated sulfonamides and increased PTs, leading to unintended hemorrhage. An algorithm from human literature was investigated and used as a template to provide a systematic process for adjusting warfarin levels, with the goals of decreasing the bleeding risk and providing guidance for adjustments. After discontinuing the use of NSAIDs and potentiated sulfonamides in the dogs receiving warfarin and utilizing the adjusted algorithm for warfarin administration, the incidence of acute bleeding episodes was mitigated. To date, 4 additional dogs have been implanted and treated with warfarin with no severe complication. Only 1 dog has presented with a minor acute bleeding episode that did not require treatment with vitamin K.

### **P37 Onsite Lateral Flow Test Device with Improved Sensitivity for Mouse Norovirus**

NE Priebsch\*

Fenway Bioscience, Inc, Boston, MA

Mouse norovirus (MNV) is the most prevalent enzootic virus in mouse colonies in bioscience. Eradication of this virus requires cost effective methods

for detection and rederivation of mouse strains. A previously developed lateral flow test for MNV was adapted to a dual pathway test platform that isolated and optimized target antibody capture from a test sample. Twenty-two test samples that were negative titer, low titer, and high titer for MNV by immune-fluorescent assay were tested. Results with dual pathway lateral flow test devices demonstrated 100% correlation with optical density results. Two samples demonstrated low immune-fluorescence and had to be retested by a more sensitive assay to confirm the presence of MNV antibody. These same samples were clearly positive with the dual pathway test devices. The reason for the improved sensitivity of the dual pathway test device was the visible, linear capture, and labeling of the antibody versus the cumulative detection of immune fluorescence on scattered colored beads suspended in liquid test media. The dual pathway test device uses 2 drops of previously diluted whole blood and gives results in 15 min. The device is intended to be used in the animal room within seconds of collecting and diluting a whole blood sample. The dual pathway test device allows real-time management of MNV-infected animals in mouse colonies.

### **P38 Onsite Lateral Flow Device for Detection of Antibodies to Five Viruses in Simians**

NE Priebsch\*

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The National Institutes of Health, Europe, and the world scientific community have adopted a standard that absence of 4 simian viruses—Herpes B, SIV, STLV, and SRVD—and positive titer for measles (rubeola virus) constitute specific pathogen-free (SPF) status for individual animals among macaques. These species constitute the most commonly used nonhuman primates in bioscience. Immunologic tests to detect antibodies for each target virus individually were adapted to a dual pathway, lateral flow test device. Purified viral lysates and recombinant proteins representing viral glycoproteins and/or viral nuclear proteins were used as antigens to produce capture lines for each of the target antibodies. Individual tests were optimized and then combined into a single test device that could detect the presence of specific antibodies to all 5 viruses simultaneously. Testing was performed with mixtures of various positive antisera. The separation of test sample flow to the antibody capture lines and optimization of individual tests allowed maximum sensitivity. This dual pathway lateral flow test device uses 1 drop of diluted whole blood and is completed in 15 min. Visual endpoint readout can be augmented using a simple device reader that gives numerical optical density readout. The device is intended to be used in the field or in an animal room within seconds of collecting a whole blood test sample. Use of this test device allows real time release, segregation, or isolation of simians in order to control potential for epizootics or maintain SPF status of colonies of animals.

### **P39 Foreign Body Rhinitis and Stunted Growth in a Mouse with Spina Bifida**

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Foreign bodies are a possible differential diagnosis for cases of rhinitis; however, there are very few reports of this condition in laboratory rodents. A 9-mo-old female transgenic mouse (kinky mutation; C57Black6/129SVS6 background) was presented with a history of respiratory noises occurring during the cage transfers. It was smaller than healthy cagemates, but otherwise seemed healthy. The tail showed an evident deformity with rigidity and vertebral deviations. Colony mice were housed under conditions compliant with Canadian animal care guidelines. Sentinel animals were negative for serology and pathology. After the external examination, the mouse was euthanized by pentobarbital overdose. The only significant gross anatomic finding was a dilation of the ileo-colic portion of the intestine. Selected abdominal and thoracic organs were taken for histology. No abnormal findings were seen in thoracic and abdominal organs except for a reduced epithelium and very thin muscular layers of the intestines. Following decalcification and histologic processing, local inflammation was seen in the nasal cavity and plant-like material (cotton) was observed. Lumbar vertebrae were incompletely formed, which could correlate with intestinal dilation, suggesting poor neural innervations of the intestines, which could be related to the stunted growth. Since the rhinitis was most likely associated with the nesting material, it was suggested that it should be removed from cages. Other microbiologic causes are more frequently associated with

rhinitis; however; our findings suggest that foreign bodies should be part of the differential diagnosis.

#### **P40 Myocardial Fibrosis in an Adult Virginia Opossum (*Didelphis virginiana*)**

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Opossums used in research have been mainly used in gastroenterology and reproductive studies. The present case is a report of an adult, 5-y-old male Virginia opossum (*Didelphis virginiana*) in severe dyspnea exhibiting open mouth breathing. Over 7 mo the animal had decreased appetite, constant weight loss, somnolence, and mild episodic dyspnea. One month prior to presentation, an episode of moderate dyspnea and open mouth breathing had occurred but had resolved spontaneously. Radiographs indicated the presence of thoracic and abdominal effusion, pulmonary atelectasis, and dilated intestines. Upon extraction of the pleural liquid via thoracocentesis the animal expired. A complete necropsy was performed. During gross examination, the right ventricular wall was noticeably thinner than normal; however, the left ventricle was normal. No other anomaly was noted. Following histologic examination, the only pathologic finding was diffuse myocardial fibrosis in the ventricles. Evidence of congestive heart failure in the opossum has been noted in several long term studies. Hubbard et al reported that in a study of 150 short-tailed opossums (*Monodelphis domestica*), pathologies of the cardiovascular system ranked third in the total number of diagnoses and ranked second as the cause of death. Myocardial fibrosis has been reported in humans and in primates and its development has been linked to excess dietary salt intake and hypertension; however, the mechanism by which these factors precipitate cardiac fibrosis is unclear. To our knowledge this is the first reported case of myocardial fibrosis in *Didelphis virginiana*.

#### **P41 Design of a Small Animal Surgical Suite**

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Surgical animal models are a vital component of biomedical research. A well-designed surgical suite is essential for producing consistent animal models. Our objective was to design a rodent surgical suite that enabled us to perform multiple rodent surgeries, while still using strict aseptic procedure and anesthetic gas delivery, with patient recovery in a temperature-controlled environment. Improving ergonomics for our staff was an additional consideration. The surgery suite was designed to support 6 surgery stations simultaneously, along with a separate prep station accommodating up to 4 rodents. Each station was designed with its own gas anesthetic delivery system featuring active evacuation of waste gas. Special surgical lighting, independent heat source, bead sterilizer, storage area, waste, and sharps containers were all incorporated into the design of each surgery station. A heated and ventilated ICU unit was selected for rodent recovery. The redesign of our surgical facility has resulted in improved efficiency, animal model consistency, postsurgical recovery as well as employee safety and ergonomics.

#### **P42 Identifying the Body Parts of Nonhuman Primates**

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The inability to accurately identify body parts can lead to numerous complications and misdiagnosis. At our facility one of the main duties of the technician is to report accurate daily observations of the animals. At times an incorrect observation of a particular body part has been reported. Accurately identifying body parts is critical information in science and medicine, particularly when describing the location of a problem or defining an area for a procedure. The primary purpose of this presentation is to help technicians learn and recognize the common and proper medical names in relation to nonhuman primates. In addition, this abstract will add to the currently available resources by showing the anatomy of nonhuman primates we commonly use in the biomedical field such as the cynomolgus (*Macaca fascicularis*). The illustrations will lay out basic external, internal,

and skeletal anatomy. Typically, the gorilla and chimpanzee views are more commonly available resources.

#### **P43 Urinary Catheterization of Male Rabbit: Evaluation of an Effective Technique and Relevant Urogenital Anatomy**

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Rabbits are widely used as models for urinary tract infection and catheter studies. However, urinary catheterization of male rabbits can be a challenge due to the anatomy of the seminal vesicular sac (SVS) opening into the urethra. In up to 40% of the cases in New Zealand white rabbits, the urinary catheter is misplaced in the SVS. Once positioned in the SVS, repositioning into the bladder is an extremely difficult task, with a high rate of failure. A modified "digital pressure" catheterization technique was developed for successful urinary catheterization of the male rabbit. The digital pressure technique enabled significantly higher catheterization success compared to the current approach. Repositioning of the catheter tip from the SVS to the urinary bladder was demonstrated using computed tomography (CT) imaging. A retrospective statistical analysis done on 45 rabbits used for urinary catheterization studies showed significant improvement in the success rate of catheterization using the digital pressure technique. We also present the relevant gross and histologic anatomy of the urogenital system of the male rabbit.

#### **P44 Nonbronchoscopic Technique for Performing Bronchoalveolar Lavage in Nonhuman Primates**

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Bronchoalveolar lavage (BAL) is a valuable procedure for diagnostic purposes for a wide range of pulmonary diseases. BAL sampling has also been an extremely useful research tool for decades and has become a commonly performed procedure on many laboratory animal species. BAL sampling on nonhuman primates is often performed with a flexible bronchoscope, which requires specialized equipment that is costly and requires significant time to clean, disinfect, and maintain. A nonbronchoscopic BAL sampling technique using inexpensive and readily available materials was developed and refined to collect alveolar macrophages from nonhuman primates on a variety of research protocols supporting the National Institute of Allergy and Infectious Diseases. The technique described reduces the overall amount of time required to perform this procedure and provides similar results in terms of cellular yield compared to using a flexible bronchoscope.

#### **P45 Facial Bleeding: Techniques and Effects**

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Blood collection from the submandibular vein (commonly known as "facial bleeding") has been described as an alternative to retroorbital bleeding, which is aesthetically unpleasant and carries the risk of ocular injury. We describe the technical aspects of facial bleeding as well as materials needed, use of anesthesia, and recognition of anatomic landmarks. Six groups of animals were bled by facial technique and were sacrificed at days 1, 2, 4, 7, 14, and 21. One group was bled weekly for 3 wk and sacrificed at day 21. Postmortem inspection of the sampling site was done and the red blood cell indices were measured and will be discussed. Facial bleeding is a viable alternative to retroorbital bleeding, which carries risk of secondary ocular injury. We hope to promote the merits of facial bleeding for regular blood sampling and to encourage more animal users to adopt this blood collection technique (we have also used this method to bleed tree shrew pups) as a refinement to other methods.

#### **P46 Reversible Contraceptive Methods Used in the Chimpanzee**

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Since 1995, a moratorium has been in effect regarding the breeding of federally owned chimpanzees. The National Center for Research Resources believed that an adequate supply of chimpanzees existed, which was being maintained for both current and future research. This decision called for a means of reversible contraception rather than permanent reproductive sterilization of the breeding colonies, taking into consideration the fact that the chimpanzees must retain the ability to breed should future research demand an increased number of these animals. A chimpanzee's life can span over 50 y, calling for a lifetime care commitment. In addition, these animals are 98.2% genetically identical to humans, underlining the importance of this animal model in the scientific advancement of improved health care for humans. Our facility houses these chimpanzees, giving special consideration to their behavior and social development and thus requiring the grouping of males and females in multi-male and multi-female groups. Various reversible contraception devices and methods are used, including intrauterine devices such as copper IUDs and levonorgestrel-releasing intrauterine systems, subcutaneous etonogestrel implants, and orally administered norgestrel and ethinyl estradiol tablets (oral birth control). For continual evaluation of the effectiveness of our birth control program, urine is collected every 60 d for detection of any unexpected pregnancies. In conclusion, these methods of contraception have proven to be effective over the past 14 y. This study serves to provide information for both veterinarians and medical staff on the benefits and failures of reversible birth control devices and methods as administered to chimpanzees.

#### **P47 Molar Malocclusions in the Laboratory Pine Vole (*Microtus pinetorum*)**

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This report describes 5 cases of molar malocclusions in adult laboratory pine voles (*Microtus pinetorum*) used for behavioral endocrinology studies. This species belongs to the subfamily Microtinae, which possesses aradicular hypsodontic molars. A total of 5 adult voles ranging in age from 9 to 18 mo of age and averaging 16.9 g were reported to the attending veterinarian for poor body condition and apparent weight loss. Upon examination, the animals' condition was determined to warrant euthanasia. Postmortem examination of the oral cavity revealed grossly elongated molars in all 5 animals. Diagnoses of molar malocclusion were made as a consequence of these gross necropsy observations and upon comparison with the literature. Four of the 5 animals in the colony identified at necropsy to have molar malocclusions had mandibular molar defects, 3 had maxillary defects, and 1 demonstrated tongue entrapment by mandibular molars. No other necropsy findings were observed. Postmortem examination of the oral cavity revealed grossly elongated mandibular and maxillary molars with improper occlusal surface wear and growth in different directions. There are few reports of dental abnormalities in Microtines. For example, bilaterally maloccluded maxillary molars have been reported in a Japanese field vole (*Microtus montebellii*), and free-ranging long-tailed voles (*Microtus longicaudus*) have been reported with molar and incisor malocclusion. Although periodontitis and malocclusion have been diagnosed in montane and prairie voles, no dental abnormalities have been reported for *Microtus pinetorum*. Consequently, we believe this is the first report of any dental abnormalities in the pine vole. This colony health problem was successfully addressed by adding autoclaved hardwood sticks to each cage as an enrichment tool to effect improved molar occlusal wear.

#### **P48 Continued Improvement of Rodent Health Problem Identification and Resolution**

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Several years ago, we used Toyota Production System (TPS) tools to develop a faster, less expensive, and more effective approach to identify and report rodent health problems. This approach was based on empowering research animal specialists (cage-front animal care personnel; RAS) to diagnose

common spontaneous illness and lesions, send the pertinent templated email alert directly to the designated research contact, and implement a standardized treatment plan developed by staff veterinarians. This program increased the response rate from investigators from 50% to 90%, reduced the average response time from 4 to 5 d to less than 1 d, and shortened the maximum delay before treatment from 12 to 3 d. In accordance with the TPS principle of Kaizen ("continuous improvement"), we sought ways to further enhance this process. A value stream manager was appointed who convened meetings with RAS and veterinarians to identify gaps and possible improvements. Observations from semiannual IACUC inspections and an AAALAC site visit were also used. Our conclusion was that while animal care staff were greatly empowered, participation of facility veterinarians was inadvertently reduced, leading to unnecessarily prolonged treatments or lapses in resolving clinical cases after they were documented. We increased communication between RAS and veterinarians by holding weekly vet rounds and also refined treatment standards to include routine veterinarian evaluation of active cases. These improvements reduced the number of long-term cases by 90%, as well as reduced the maximum treatment time from more than 3 mo to 1.

#### **P49 The Tail of the Rat-King Phenomenon**

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Widely thought of as a crypto-zoological phenomenon, Rat-King stories have come to be seen as folklore, not as an actual biologic occurrence. Few cases have been documented, the earliest in the late 1500s and the latest in 2005 in Estonia. The most famous Rat-King was found in Germany in 1828. It consists of 32 mummified rats and is on display in the Mauritium Museum in Altenburg, Germany. Our institution discovered a small scale "Mouse-King." In a static microisolation cage of 5 female adult Balb/c mice used for an immunology experiment, an animal care technician observed that 4 of the 5 mice had their tails tied together in a knot. Upon closer inspection, the tails appeared braided together. All mice were bright, alert, and responsive. The animals were anesthetized and the tails were humanely unbraided to free each individual mouse. One mouse did not recover from anesthesia. All others recovered and healed without complication. We speculate that this may have been caused by huddling, inactivity, or injuries that caused the tails to become sticky and adhere. However, it is impossible to know the exact cause. Although rare, laboratory mouse caregivers need to know that this condition exists and if it goes unrecognized, animals with braided tails may suffer dehydration and die.

#### **P50 Adverse Effect of Supplemental Heat in Lipopolysaccharide-Treated Mice**

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Clinical supportive care is offered for rodents on research studies to prevent or alleviate adverse clinical signs associated with experimental treatment. Supplemental heat (provided by heated rack or circulating water bath) and moistened ground feed (mash) provided in a petri dish in the cage are 2 of the supportive measures commonly offered. Lipopolysaccharide (LPS) is a commonly used experimental agent to induce an inflammatory response; mice appear lethargic within 4 h of LPS treatment, but recover by 48 h. Supplemental heat was added as many of the animals treated with LPS appeared hypothermic. The heat did not improve clinical signs, and in some cases, mice became more lethargic when placed on supplemental heat. To objectively evaluate supplemental heat as clinical support for LPS-treated mice, group- and singly housed mice were dosed with LPS (2 mg/kg) or saline intraperitoneally, and cages were either placed on a heated shelf (supplemental heat) or a nonheated shelf on the same rack. The temperature of an empty cage on each shelf was monitored. All mice were provided with mash, prepared 1:1 with reverse osmosis deionized water, which was helpful in maintaining the hydration and nutritional status of the animals during this 48-h period. Body temperature, weight, and serum corticosterone levels were measured and documented during the 48-h period. Body weights decreased in all LPS-treated mice. Body temperatures dropped more dramatically in the single housed animals than the group housed mice 4 to 12 h after dosing with LPS; however, returned to baseline by 24 h. Mice dosed with LPS and given heat had

serum corticosterone levels significantly higher at 12 h compared to the LPS-treated mice receiving no heat, suggesting that heat is contraindicated as a supportive measure in animals treated with LPS.

### **P51 Alternative Methods for Placing ECG Leads in Rats to Achieve a Better P Wave**

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The P wave represents the wave of depolarization that spreads from the sinoatrial (SA) node throughout the atrium and is usually 0.08 to 0.1 s (80 to 100 ms) in duration. If the P to R interval, which is the period of time from the onset of the P wave to the beginning of the QRS complex, is less than 0.2 s, then there is an atrioventricular (AV) conduction block. AV is also known as a first-degree heart block if the impulse is still able to be conducted into the ventricles. The unit used is a small animal radio telemetry transmitter, which is capable of continuously monitoring blood pressure and ECG simultaneously for at least 2 mo (longer if the transmitter is turned off when not being monitored). There are less invasive methods of measuring blood pressure and ECG available, but they do not offer the capabilities of continuous monitoring of unrestrained rats. The transmitter has a catheter that is inserted into the descending aorta and 2 ECG lead wires, one positive and the other negative. Traditional placement of the ECG lead wires is to attach the positive (red) lead wire subcutaneously approximately 1.5 to 2 cm left of the xyphoid process and to attach the negative (clear) ECG lead wire beneath the pectoral muscle on the right side of the animal's chest. Placing the ECG lead wires in this manner led to a lot of "noise" from the animals' movements, which prevented us from seeing the P wave, making it difficult to read the QRS complex. The alternative placement method for the positive and negative leads is to attach the positive ECG lead wire on the dorsal side of the xyphoid process and to attach the negative ECG lead wire under the sternohyoid muscle to lay directly on top of the trachea near the thoracic inlet. With this method we were able to reduce the amount of noise produced with the traditional method.

### **P52 Gross Renal Involvement of Polyarteritis Nodosa in a Rat with Concurrent Chronic Progressive Nephropathy**

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A 12-mo-old female rat presented with bilateral periorbital porphyria, lethargy, and chronic wasting. The rat was generated by crossing an L1 strain (transgenic for a human *L1* gene encoding a transposon) with a Sprague-Dawley strain to better characterize the genetic process of retrotransposition. Following euthanasia, a thorough necropsy was performed and a complete set of tissues was evaluated microscopically. The right kidney, weighing 3.26 g, was 75% larger than the left kidney, which weighed 1.86 g. In addition, the enlarged right kidney contained multiple, coalescing, bulbous swellings that elevated the anterior two-thirds of the lateral renal capsule. The remaining surface of the right renal capsule and the entire left renal capsule were moderately pitted. The entire intestinal mesentery contained many individual and coalescing plum-colored nodules. The nodules were tortuous and paralleled the radiating vasculature in the mesentery. Microscopic analysis revealed that the bulbous swellings in the right kidney were thickened, dilated, thrombotic, renal arteries with fibrinoid degeneration of the tunica media. The pathologic changes of the right kidney were characteristic of polyarteritis nodosa, which was confirmed in the mesentery and was also found microscopically in the cerebrum, myometrium, and mesometrium. In addition to polyarteritis nodosa, microscopic lesions characteristic of chronic progressive nephropathy were noted in both kidneys. This is the first reported case of gross lesions of polyarteritis nodosa in the rat kidney.

### **P53 Stomach-36 Acupuncture as Adjunct Therapy for Debilitation and Anorexia in Cynomolgus Macaques (*Macaca fascicularis*)**

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Supportive therapy for debilitated or anorexic animals is a challenge. Acupuncture is used in supportive care for animals as well as people. In particular, an acupuncture point on the lower leg called Stomach-36 or zusanli is used as a nonspecific stimulant of appetite and wellbeing. A group of adult cynomolgus macaques (*Macaca fascicularis*, Mauritius origin) recently shipped to the primate quarantine holding facility developed diarrhea, precipitous weight loss, anorexia, and dehydration. Several animals died despite aggressive critical care therapy, which included serial diagnostic laboratory tests, IV fluid therapy, SC fluid therapy, antibiotics, heat support, gastric protectant drugs, and oral gastric tube feeding of food and electrolytes. Necropsy findings included intestinal nematodiasis, balantidiasis, and amoebiasis. Five animals in the group had been inappetent for 3 d and had lost substantial weight in spite of medical treatment. After stimulation of acupuncture point Stomach-36, all of the animals in the treatment group responded with markedly increased appetites within 12 to 24 h, although 1 animal subsequently died. This presentation illustrates the technique for Stomach-36 acupuncture. The use of Stomach-36 as adjunct therapy should be considered as part of a complete treatment plan for stock monkeys that are debilitated or have gastrointestinal disturbance. The animals in the present case were recently acquired and were not on studies. Acupuncture was useful for their treatment; however, because of the potential for poorly understood physiologic and medicinal effects, we do not advise the use of acupuncture on animals being used for regulated drug studies.

### **P54 Rapid Detection of Murine Gastroenteric Bacteria Using Multiplex PCR Assay**

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*Helicobacter hepaticus*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium* were responsible for gastroenteric diseases in rodent. The accurate and economical diagnosis of pathogenic bacteria is necessary for microbiologic control of laboratory animals. In this study, multiplex PCR method was developed for simultaneous detection of 3 common gastroenteric bacteria in rodent. The target nucleic acid fragments were specifically amplified by consensus PCR analysis with 16S ribosomal RNA of *H. hepaticus*, complete genome of *P. aeruginosa*, and *invA* gene of *S. typhimurium*. To investigate the specificity of primers, they were tested against DNAs of other bacteria. There was no amplification from other bacteria such as *CAR bacillus*, *Corynebacterium kutscheri*, *Klebsiella pneumoniae*, *Mycoplasma pulmonis*, *Pasteurella multocida*, *Pasteurella pneumotropica*, and *Streptococcus pneumoniae*. The target genes (417, 724, and 246bp) were specifically amplified by PCR with *H. hepaticus*, *P. aeruginosa*, and *S. typhimurium*. Serial 10-fold dilutions of each bacterial DNA were tested with our multiplex PCR assay. The detection limits of multiplex PCR assay were 50 pg, 10 pg, and 50 pg for the *H. hepaticus*, *P. aeruginosa*, and *S. typhimurium*, respectively, in pure cultures. And *H. hepaticus*, *P. aeruginosa*, and *S. typhimurium* were simultaneously detected in feces, cecum, and liver of mice infected by single or multiple pathogens using multiplex PCR. This multiplex PCR assays will be a useful and convenient method for the rapid identification of bacterial pathogens from laboratory animals. Additionally, our PCR method will be used to eradicate intestinal bacterial infection in laboratory animal facilities and for quality control of laboratory animals.

### **P55 Multiplex PCR assay for Rapid Identification of *Klebsiella pneumoniae*, *Pasteurella multocida*, and *Streptococcus pneumoniae***

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*Klebsiella pneumoniae*, *Pasteurella multocida*, and *Streptococcus pneumoniae* are important pathogens, which cause respiratory infection in laboratory animals. In this study, we investigated the usefulness of species-specific PCR analysis to detect 3 common bacterial pathogens related with respiratory diseases. The target nucleic acid fragments were specifically amplified by consensus PCR analysis with *waa* gene of *K. pneumoniae*, *sodA* gene of *P. multocida*, and *lytA* gene of *S. pneumoniae*. The target genes (618, 409, and 264bp) were specifically amplified by PCR with *K. pneumoniae*, *P. multocida*, and *S. pneumoniae*. To investigate the specificity of primers, the primers were tested against DNAs of other bacteria. There was no amplification from other bacteria such as *CAR bacillus*, *Corynebacterium kutscheri*, *Helico-*

*bacter hepaticus*, *Mycoplasma pulmonis*, *Pasteurella pneumotropica*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. The target gene was specifically amplified by PCR for *K. pneumoniae*, *P. multocida*, and *S. pneumoniae*. Serial 10-fold dilutions of each bacterial DNA were tested with our multiplex PCR assay. The detection limits of multiplex PCR analysis in pure cultures were 10 pg, 10 pg, and 50 pg for the *K. pneumoniae*, *P. multocida*, and *S. pneumoniae*, respectively. And *K. pneumoniae*, *P. multocida*, and *S. pneumoniae* were successfully detected in lung, trachea, and nasal swabs of mice infected by single or multiple pathogens using multiplex PCR analysis. This multiplex PCR analysis will be a useful and effective method for simultaneously detection of *K. pneumoniae*, *P. multocida*, and *S. pneumoniae* in various kinds of laboratory animals including rodents. Additionally, our PCR method will be used to eradicate respiratory bacterial infection in laboratory animal facilities and for quality control of laboratory animals.

#### **P56 Comparison of Blood Vessels of the Mouse and Rat Tail Using Angiography and Histology**

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Values obtained from blood are used as important components of scientific research, including in pharmacology and physiology. Although there are many sites for collecting blood in mice and rats, the tail vessels and orbital sinus are frequently used for serial blood sampling. We evaluated the possibility that blood collected from the tail vein could lead to the mixing of arterial and venous blood. First, we investigated how many arteries existed in the tail of 5 C57BL/6J mice and 4 Sprague-Dawley rats using angiography with fluoroscopy and radiography. Radiographs showed a radiopaque line as an artery running in the middle ventral portion in the mouse and rat tail. Interestingly, a radiopaque line as ventral vein was observed dorsal to midventral artery in the rat tail. Especially in the rat, fine arterioles branched upward from the artery on the lateral and ventrodorsal view of angiographic images. Next, we analyzed histologically the transverse sections of the tail of the same mice and rats. We observed that tendons and muscles surrounded by thick fascia were divided into 4 bundles, and blood vessels were nestled at grooves closed by the fascia. In addition to arterioles branching upward from the ventral artery, they were also observed at the lateral side of the mouse and rat tail. Taken together, we found 1 ventral artery, 1 dorsal vein, 1 ventral vein, and 2 lateral veins in the mouse and rat tail. However, previous reports noted that there are 1 ventral artery, 1 dorsal vein, and 2 lateral veins in the mouse and rat tail. Therefore, we must consider the possibility that tail incision could cause mixing of artery and venous blood in mice and rats.

#### **P57 A Method for Repeated Blood Sampling from the Jugular Vein of Conscious Mice**

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We developed a new method for serial blood collection from the jugular vein of mice. The jugular vein was penetrated with a 27G needle, and approximately 30 to 200  $\mu$ L of blood was collected in a 1 mL tuberculin-type syringe. Immediately after blood collection, gentle pressure was applied to the site to stop the bleeding. To validate this method of blood collection, we performed a pharmacokinetic (PK) study and an oral glucose tolerance test (OGTT) in mice. In the PK study, compound A was administered orally at 3, 10, or 30 mg/kg to ICR mice. Blood was drawn at 15, 30, 60, 120, and 240 min after the administration. OGTT was performed by the administration of glucose at 2 g/kg to C57BL/6 mice; the blood glucose levels were measured at 0, 15, 30, 60, and 120 min after the glucose load. All procedures were performed without anesthesia. The plasma concentration of compound A increased dose-dependently within 15 min and then gradually decreased thereafter. Linear increases in AUC and C<sub>max</sub> were seen with linear increases in the administered dose. In OGTT, vehicle-treated mice had a significant increase in glucose levels following an oral glucose challenge. The overall glucose excursion during OGTT was significantly reduced in mice treated with exendin-4 (5  $\mu$ g/kg IP), a GLP-1 receptor agonist. The mean time from the beginning of animal handling to the end of blood collection was within 1 min, and this technique also allowed drawing up to near the threshold of the volume of blood sampling. We further confirmed that serial blood col-

lection had caused no significant damage at the injection sites. Serial blood collection from the jugular vein may be of particular interest for studies that attempt to examine the time dependent changes in blood variables in laboratory mice.

#### **P58 The Use of the 3Rs Principle in a Study about Physiology of Stress**

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Chronic mild stress protocol (CS) induces anhedonia in laboratory rats and has been used in studies about depression and stress. To study behavioral, metabolic, and oral effects of CS in rats, observing a better application of 3Rs principle, twenty-four 2-mo-old male rats were divided into 2 groups: control (C) and CS. Body weight, food intake, and oral production of volatile sulfur compounds were analyzed weekly for 7 wk. CS was applied from weeks 3 to 5. Anhedonia was evaluated by sucrose preference test before, during, and after stress. All animals were submitted to behavioral tests for evaluation of anxiety, learning, and memory at week 6. Oral glucose tolerance test (OGTT) was done at week 7. After sacrifice (week 8), blood was collected for hormones and lipids dosages. CS group presented lower body weight gain during and 1 wk after stress, and lower final body weight. However, food intake decreased only at week 3, and increased at week 6. Higher levels of oral volatile sulfur compounds were observed in CS group from week 3 to 6. CS decreased sucrose preference, learning skills, and memory, but increased locomotor activity in comparison to C. CS rats presented higher area under curve in OGTT. Two weeks after stress, CS rats presented higher corticosterone, leptin, triglycerides, total cholesterol, LDL plasma levels, and higher atherogenic index. These data showed that CS changed oral homeostasis, behavioral responses, body weight control, glucose, and lipid metabolism, providing a broader comprehension about the effects of CS. The study was developed by 2 professors, 7 graduate and 2 undergraduate students, and 1 technician. The involvement of all the team in the planning, treatment of animals, and data analysis contributed to the refinement of scientific methodology, because the problems, doubts, and future analysis are discussed together before the experiments are made. Moreover, it also reduced and optimized the use of laboratory animals since the same animals can be used simultaneously in the projects of 2 or 3 students with cardiovascular, metabolic, and behavior analysis, for example.

#### **P59 C57BL/6NHsd Male Mice Started on High-Fat Diets at Three, Six, or Nine Weeks of Age Attain Similar Obesity Phenotypes**

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The C57BL/6 mouse strain fed a high-fat diet (HFD) with approximately 60% of kcal from fat (lard) is an established rodent model of diet-induced obesity. To determine if there is an ideal start age or diet composition that enhances development of obesity phenotypes, 2 HFD patterns were initiated at 3, 6, or 9 wk of age. The HFD varied in fat (F, amount and type) and sucrose (S), abbreviated as follows in percent energy: 45F30S (milkfat) and 60F10S (lard). The HFD fed mice were compared to mice fed a purified low-fat control (17F7S). Body weight and food disappearance were assessed once weekly in the 9 groups of mice ( $n = 15$  to 16 per group). Half of the mice had serial DEXA measurements performed at 9, 15, and 19 wk of age. The remaining mice underwent a glucose tolerance test at 17 wk of age. Within 3 to 4 wk, mice started on the HFD were significantly heavier than controls irrespective of the age the diet was started. Upon initiation of HFD at 6 or 9 wk, mice rapidly increased their rate of weight gain and attained similar weight compared to 3 wk HFD mice with the exception of 9 wk 60F10S, which failed to catch up by 19 wk. Calculated energy intake was greatest in the first week following HFD initiation. Intake tended to be higher throughout the remainder of feeding in all HFD groups. DEXA measurements at 9 wk of age revealed animals fed HFD for 3 or 6 wk showed similar percent fat that was approximately double controls (17% versus 32%). By 15 and 19 wk of age all HFD groups showed a doubling of percent fat compared to controls (22% versus 44% at 15 wk and 25% versus 46% at 19 wk). Independent of start age, GTT at 17 wk revealed HFD fed mice were glucose intolerant compared to control animals with



a 60% increase in glucose area under the curve. In summary, these 2 HFD diet patterns produced similar obesity phenotypes. The age at which these HFD were did not impact the percent fat and glucose tolerance measured after 8 to 14 wk of feeding.

#### **P60 A Diet with 45% kcal from Fat Produces Similar Obesity Phenotypes in Mice as Diets with 60% kcal from Fat**

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Teklad Diets, Harlan Laboratories, Inc, Madison, WI

Current commercially available diet-induced obese (DIO) mice are predominantly fed a high-fat diet with approximately 60% of kcal from fat (lard). In this study we compared the obesity phenotypes of C57BL/6NHsd male mice fed 3 purified high-fat diets (HFD). The high-fat diets varied in fat (F, amount and type) and sucrose (S), abbreviated as follows in percent energy: 45F30S (milk-fat), 60F15S (milkfat-lard), and 60F10S (lard). Control diets included a standard nonpurified diet (2018S) and a purified low-fat control with approximately 17% of kcal from fat, resistant starch, and minimal sucrose, approximately 7% kcal (17F7S). Barrier-reared mice (4 per cage) were fed the diets starting at 3 wk of age and weighed weekly. At 10 and 18 wk of age, 18 to 20 mice per diet were sent for subsequent phenotyping performed 1 to 2 wk later. Measurements included determination of percent body fat (%fat) by NMR, glucose tolerance tests (GTT), and fasting and nonfasting serum parameters including glucose, triglyceride, free fatty acid (FFA), and cholesterol levels. Within 2 wk of feeding all HFD produced significantly higher weight than the control diets. By 12 wk of age animals fed the HFD were approximately 30% heavier (26 versus 33 g) with approximately 90% increase in %fat (19% versus 35%) compared to controls. GTT area under the curve and fasting glucose values at 12 wk of age were significantly different ( $P < 0.05$ ) with 2018S = 17F7S < 45F30S < 60F15S < 60F10S. By 19 wk of age animals fed the HFD were approximately 45% heavier (30 versus 43 g) with approximately 90% increase in %fat (23% versus 44%) compared to controls. All 3 HFD produced similar increases in weight and %fat. Nonfasting cholesterol was higher at 12 and 19 wk of age in all HFD groups compared to controls. The 17F7S diet with resistant starch had significantly lower nonfasting glucose and cholesterol compared to all other groups. In summary, the variety of HFD compared here produce similar increases in obesity phenotype, and a purified control diet with resistant starch may be advantageous by lowering the baseline value for obesity-associated phenotypes.

#### **P61 Development of Protocols to Optimize Treatment of Spontaneous Type 1 Diabetes in BB Rats**

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Human type 1 diabetes (T1D) is reversible via islet transplant and chronic immune-suppression. However, preclinical trials are needed to develop less toxic agents. Previous studies in rodents demonstrate that intense insulin therapy preserves pancreatic beta cells. Therefore, to evaluate efficacy of new therapies in preventing/reversing diabetes we developed a standardized protocol (SP) for maintaining glycemic levels in diabetic rats. Factors considered in the development of this SP included type of insulin, dose, time of dose, frequency, and percentage of daily dose relative to fed state (lights off). We studied groups of 6 to 12 acutely diabetic BB/Wor rats. We evaluated the effect of a once a day dosing with a single insulin, protamine zinc insulin (PZI), versus BID dosing using PZI as the first insulin and different types of insulins (PZI, Humulin 50/50, or Humulin 70/30) for the second dose to determine optimum timing of dosing and type of insulin to maintain normoglycemia. Blood glucose levels were measured every 6 to 12 h for 5 d. We found the daily dose of insulin in the rat for nonintensive therapy to be approximately 0.9 U PZI/100 g of body weight (BW). We determined that an intensive insulin regime comprised of 2 injections of PZI injected at a dose of 0.7 U/100 g BW 4 h before and 0.5 U/100 g BW 8 h after lights out provided excellent glycemic control. We next extended these studies to determine whether glycemic control could be maintained for 3 wk. A group of 11 rats were treated with PZI BID dosing and blood glucose levels were measured every 12 h. We observed only minor fluctuations in glycemic levels (approximately 50 mg/dL) during this 3 wk time period. However, we noted it was necessary to adjust insulin by 0.2 U increments whenever serum glucose levels were out of the normal range established by BBDR/Wor rat controls. Use of this standard protocol should aid in the testing of agents to prevent/reverse diabetes.

#### **P62 Insulin Glargine for Long Term Treatment of Streptozotocin Induced Diabetes in a Yorkshire Pig**

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A 17.1 kg, 4-mo-old castrated male Yorkshire pig (*Sus scrofa domestica*) enrolled in a study examining mechanisms underlying diabetes accelerated atherosclerosis presented with lethargy and anorexia 21 d post streptozotocin administration. Physical exam revealed generalized muscle wasting. Lab work showed hyperglycemia (440 mg/dL) and glucosuria in the absence of ketonuria. Two units of regular insulin were administered intramuscularly, an electrolyte solution provided orally, and blood glucose monitored every 2 to 4 h. Free catch urine was sampled once daily to test for the presence of ketones. Regular insulin was discontinued, and 3 units of insulin glargine were administered subcutaneously every 24 h for 60 d, after which time insulin was discontinued due to an improved health profile. One week after discontinuing insulin, the pig presented depressed and listless. Lab work revealed hyperglycemia (327 mg/dL), elevated serum ketones (55.7 mg/dL), and ketonuria. LRS supplemented with 2.5% dextrose was administered intravenously at 60 mL/kg/d. Regular insulin was administered at 3 units IM, cephalixin at 250 mg IV q 8 h, and famotidine at 10 mg IM q 12 h. Regular insulin dose was determined after blood glucose results were obtained every 2 to 4 h. Regular insulin and fluids were discontinued and 3 units of insulin glargine restarted after obtaining negative urine ketones. Insulin glargine is a long-acting parenteral blood-glucose-lowering agent labeled for human use. The effect profile of insulin glargine is relatively constant, with no pronounced peak and prolonged duration. Its use has been reported previously in Goettingen minipigs in which diabetes was induced by streptozotocin or by surgical pancreatectomy. In that study, insulin glargine alone kept the Goettingen minipigs in good condition for 5 to 6 mo, with stable body weight and normal behavior. Alternatively, a premixed solution containing fast-acting insulin was administered concurrently with insulin glargine before feeding to improve glycemic control. Subsequently, Yorkshire pigs enrolled in the current study have received insulin glargine once daily to maintain body weight while simultaneously ensuring a stable hyperglycemia for atherosclerotic plaque formation without complication.

#### **P63 Blood Glucose Profiles following IVGTT in Two Miniature Swine Lineages**

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Diabetes mellitus is an epidemic that is increasingly prevalent worldwide, especially in children. Two main types of diabetes are recognized: type 1 (juvenile onset or insulin-dependent) and type 2 (adult onset or noninsulin-dependent) and miniature swine is becoming an increasingly recognized model. A number of standard tests are commonly used to monitor the severity of diabetes in lab animals, including OGTT, IVGTT, glucose clamps, insulin resistance, glucose intolerance, hypertension, dyslipidemia, ERG, and histology (for example, atherosclerosis); however, some tests still lack some validation in the miniature swine. We believe that a glucose dose of 600 mg/kg is appropriate for intravenous glucose tolerance test (IVGTT) in miniature swine. We measured blood glucose levels (bG; mg/dL) by glucometer at set time intervals for 120 min following rapid intravenous infusion (600 mg/kg) of glucose. Baseline bG averaged 121 in Hanford ( $n = 2$ ) and 74 ( $n = 3$ ) in Yucatan. Following intravenous infusion, bG rapidly peaked at 60 s increasing approximately 4-fold in Hanford and 6-fold in Yucatan and then rapidly fell back to normal levels within 30 to 60 min, with slight overcompensation. We can then conclude that the 600 mg/kg glucose was adequate to detect rapid and standard IVGTT bG profiles in both miniature swine lineages. The IVGTT bG profiles are clearly different between both miniature swine lineages; the IVGTT bG profile curves for Yucatan were most homogenous with the least variability between individuals.

#### **P64 Comparison of Human, Porcine, and Rodent Wound Healing With New Miniature Swine Study Data**

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Accidental wounds are one of the most common reasons for human visits to hospital emergency services. Healthy individuals normally heal accidental skin wounds in a rapid period if complications, such as infections, can be avoided. Aged individuals or diabetics frequently suffer delayed wound healing. Accidental wounds are one of the most common reasons for human visits to hospital emergency services. Healthy individuals normally heal accidental skin wounds in a rapid period if complications, such as infections, can be avoided. Aged individuals or diabetics frequently suffer delayed wound healing. Wound researchers require efficient animal models that are predictive of human responses. Pig skin is anatomically, physiologically, biochemically, and immunologically similar to human skin, and the skin is "fixed skin" like humans and unlike rodents or rabbits. Loose skin allows accelerated closure of surgically induced rodent wounds by primary contraction unlike the normal primary response of reepithelialization in swine and humans. Pig skin mirrors human skin in having a sparse haircoat, a relatively thick epidermis, similar epidermal turnover kinetics, lipid composition and carbohydrate biochemistry, lipid biophysical properties, and a similar arrangement of dermal collagen and elastic fibers. In our studies, healthy juvenile miniature swine wound reepithelialization progressed relatively quickly (average 0.109 mm/h at day 19) while geriatrics progressed more slowly (average 0.048 mm/h at day 32). Untreated adult diabetic Yucatan miniature swine wound reepithelialization rates were 88% of conventional nondiabetic (control) Yucatan models at day 29 after wounding. Postulations for this minor difference will be offered. Control juvenile or young adult Sinclair or Yucatan miniature swine dermal wounds heal at faster rates (reepithelialization rate) than those of geriatric or diabetic animals. The healing rate differential can be as high as 2:1 for juveniles over geriatrics. Porcine or miniature swine models offer significant advantages and have a record of predicting treatment modalities in human over models with loose skin. Miniature swine models can provide useful efficacy data for novel cutaneous therapy products.

#### **P65 Feeding Extract of *Salacia oblonga* in Rice Ball Meal Lowers Blood Glucose in Insulin-Resistant Rats**

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*Salacia oblonga* has a long history of use and a traditional reputation as a treatment for diabetes. *Salacia oblonga* extract (*Salacia*) works as an inhibitor of starch digestion which is normally assessed by a meal tolerance test. In animal studies, *Salacia* is delivered in a polysaccharide (maltodextrin) vehicle. We wanted to see if the effects seen there generalize to foods normally consumed by humans. Rice balls are an excellent way to deliver a meal in a more physiologic way during a meal tolerance test. To evaluate the effectiveness of *Salacia* ingested with a solid meal, 20 male Zucker fa/fa rats (9-wk-old) were trained to eat rice by offering small amounts of rice daily and increasing the amount until they could consume a 4 g rice ball within 5 min. On the test day, overnight fasted rats were divided into 2 groups matched by body weights (Mean  $\pm$  SEM) Control: 447  $\pm$  9 and *Salacia*: 435  $\pm$  10 g. One group received 4 g of cooked rice with a dose of 25 mg/kg body weight of *Salacia* added; the other group received 4 g of plain cooked rice as a control. Blood glucose was measured with a blood glucose testing system before feeding and afterwards at 30 min intervals for 2 h. There was no significant difference in blood glucose between the groups before the meal (Mean  $\pm$  SEM) Control: 106.6  $\pm$  4 mg/dL, *Salacia*: 107.8  $\pm$  8 mg/dL. Feeding *Salacia* in rice significantly ( $P < 0.05$ ) decreased blood glucose levels at 30, 60, and 90 min compared to control. *Salacia* also decreased the positive adjusted area under the glucose curve by 40.8% compared to the control. These results are comparable to a previous study where a 25 mg/kg body weight dose of *Salacia* was delivered by gavage in a maltodextrin vehicle. *Salacia* significantly ( $P < 0.05$ ) decreased blood glucose levels at 30 and 60 min compared to control and also decreased the positive adjusted area under the glucose curve by 29.2% compared to the control.

#### **P66 Absorption of Insulin into the Portal Vein and Peripheral Blood after Intraintestinal Application**

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Oral administration of insulin may offer the advantages of convenience and patient compliance and resemblance to normal physiology with introduc-

tion of insulin into the portal vein and extraction in the liver. To improve transport and absorption of biologically active insulin from the intestinal lumen into the blood circulation, insulin must be protected against proteolysis and the transport must be enhanced to allow absorption of a significant and measurable amount. The aim of this study was to establish a simple in vivo model in anesthetized rats to determine insulin levels in portal and peripheral blood, and to estimate the hepatic extraction after application of a human insulin analogue into the small intestine. Male Sprague-Dawley rats fasted for 18 h were anesthetized subcutaneously with fentanyl/fluanisone/midazolam and catheters were inserted into the portal vein, small intestine (jejunum, 50 cm proximally to caecum), and right jugular vein, respectively. Rats were randomly assigned to low or high human insulin analogue doses; 50 nmol/kg (LD,  $n = 7$ ) or 150 nmol/kg (HD,  $n = 6$ ). Dose volume was 0.4 mL/kg. Blood samples were collected regularly for 3 h for the determination of blood glucose and plasma insulin. The total clamping time of the portal vein was 1.4  $\pm$  0.1 min (Mean  $\pm$  SEM). Portal insulin peak values were 722  $\pm$  247 pM (LD) and 1686  $\pm$  622 pM (HD). Peripheral insulin peak values were 415  $\pm$  122 pM (LD) and 1054  $\pm$  312 pM (HD). T<sub>max</sub> was at 30 min. The estimated hepatic extraction calculated from the portal and peripheral insulin AUCs was 22%  $\pm$  10 (LD) and 29%  $\pm$  7 (HD). The model allows a fast estimate of the extraction of a given insulin analogue after intestinal absorption. Further model development including cannulation of the hepatic vein would facilitate more accurate assessment of first-pass hepatic extraction.

#### **P67 The Zucker Rat: A Model for Diabetic Renal Nephropathy**

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Diabetes is the fifth deadliest of US diseases, with an economic cost (2002) of US\$132 billion. Approximately 30% to 40% of diabetics develop renal nephropathy with greater numbers among African Americans, Hispanics, and Native Americans, suggesting ethnic/genetic influences on susceptibility. Diabetes models are as complex as the human condition and differences in physiologic response create difficulty in evaluating relevant pathology. In a 13-wk toxicity study, lean and obese Zucker fa/fa rats were obtained from 2 commercial vendors and evaluated from 8 to 20 wk of age. While gram body weight did not differ significantly between the rats (vendor A lean 251  $\pm$  13, obese 360  $\pm$  13; vendor B lean 256  $\pm$  5, obese 389  $\pm$  9 at 8 wk), clinically significant differences occurred. At 18 to 20 wk, serum triglycerides of vendor A lean Zuckers exceeded those of vendor B (106.4  $\pm$  14.0 versus 61.1  $\pm$  7.9 mg/dL, respectively,  $P < 0.02$ ) as did leptin (10.1  $\pm$  1.1 versus 3.9  $\pm$  0.6 ng/mL, respectively,  $P < 0.001$ ). Vendor A obese Zuckers exceeded those of vendor B: serum cholesterol (505.0  $\pm$  88.3 versus 248.5  $\pm$  13.3 mg/dL, respectively,  $P < 0.02$ ), triglycerides (1456.2  $\pm$  388.4 versus 480.5  $\pm$  35.7 mg/dL, respectively,  $P < 0.03$ ), glucose (304.6  $\pm$  22.7 versus 239.5  $\pm$  20.1 mg/dL, respectively,  $P < 0.05$ ), and leptin (43.6  $\pm$  5.5 versus 30.8  $\pm$  2.1 ng/mL, respectively,  $P < 0.05$ ). At 18 wk, urinary protein concentration was 6-fold higher in vendor A lean Zuckers compared to vendor B (210.7  $\pm$  182.6 versus 44.8  $\pm$  28.5 mg/dL, respectively). Renal nephropathy, evaluated at 20 wk, correlated with urinary protein concentrations and was more severe in obese rats from vendor A than from vendor B. Rats from both vendors developed skin lesions associated with *Staphylococcus aureus* infection, but the condition was more severe in rats from vendor A. Observations suggest that diabetes and related pathologies develop at a faster rate in Zucker rats from vendor A than in those from vendor B.

#### **P68 Pink Lab Coats Improve Lab Coat Retention and Compliance with Personal Protective Equipment Policy**

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The PHS Guide requires laboratory animal facilities to provide protective clothing, such as lab coats, and laundry services to all animal users and staff to reduce the spread of pathogens through contaminated clothing. Lab coat policies are most effective if personnel wear only facility-provided lab coats while in the animal facility and dedicated lab coats are readily available to all personnel. However, compliance is difficult if there is repeated loss of reusable lab coats due to removal from the facility. We hypothesized that

pink-colored lab coats would discourage removal and wearing of the lab coats outside of the animal facility and thus decrease the need to replace lab coats. For a 6-mo period, pink cloth lab coats replaced white cloth lab coats at the main entry into the facility. The department purchased all lab coats, dyed them pink, and provided in-house laundry services. The average monthly number and cost of lab coats purchased were compared before and after the color change. In addition, the cost and time spent laundering lab coats were monitored. The increased retention of the pink lab coats compared to the white lab coats was noticed almost immediately. The total number of lab coats purchased decreased by 64% (1,080 versus 390) over a 6-mo period. Overall, cost savings realized were US\$1,701 (US\$2,811 versus US\$1,110), representing a 60.5% decrease in the cost of lab coats over the same period prior to the color change. Changing the lab coat color from white to pink discouraged the removal of lab coats from the facility, thereby decreasing costs associated with this important personal protective equipment item and potentially decreasing the spread of pathogens into and outside of the facility.

#### **P69 Rabbit Acclimation Program: A Portrait of Love and Care**

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A rabbit has an instinctive defensive reaction in situations they perceive as threatening. In a laboratory setting, these threatening situations can be defined as a novel setting, physical restraint, or undergoing an unfamiliar procedure that may produce stress, which could then affect the outcome of an experiment. To help dissipate the rabbit's natural instinct to flee from its predators and to help encourage the rabbit's increased interaction, a program of regularly scheduled acclimation involving 64 rabbits was implemented at the end of January 2009. Upon their arrival in the vivarium, naïve rabbits are allowed to acclimate in their cages for a period of time of no less than 1 wk. During this time no handling other than routine cage changing or required veterinary care is performed. After this initial preconditioning period, the rabbits are then acclimated using a thrice-weekly socialization regimen, with each day consisting of a morning and an afternoon session. During the morning session each rabbit is removed from its cage by an appropriately trained technician and, using proper restraint techniques, the rabbit is held for 5 min. During the afternoon session each rabbit is removed from its cage and allowed to sit in a restrainer for 5 min. While in the restrainer rabbits are not to be left unattended. After returning the rabbit to its cage, the technician can provide a treat to the animal. The SOP for rabbit acclimation has recently been updated to provide a more flexible schedule that technicians can adjust to better meet each rabbit's individual acclimation needs. All acclimation activities are recorded on a weekly acclimation worksheet. Feedback from both husbandry and research staff indicate a general reduction in stress associated with handling and procedural manipulations in rabbits that received regular acclimation. The reduction of stress in rabbits improves productivity, lessens time needed to perform studies, and increases data quality.

#### **P70 Investigating Enrichment Preferences for CD-1 Mice Housed on Wire-Bottom Caging**

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Marbles have been one of the standard enrichment items for CD-1 mice housed in wire bottom cages at our institution. Although the industry standard for rodent caging has shifted to shoebox caging, wire bottom cages are necessary for use on particular toxicology studies. Recent research has shown that marbles used as enrichment with mice are stressors and the best type of enrichment items are those that encourage natural behaviors, such as nesting and gnawing. Based on published research, we investigated possible alternatives to our standard enrichment by evaluating mouse preferences for readily available enrichment items. Mice were given a variety of items (that is, houses, nesting materials, and chew toys) over the course of 2 mo and their use of these items was monitored. Usage was determined by the following indicators: sleeping in the houses, nesting, or gnawing on the chew toy, and to what degree and length of time the items were used. Results demonstrated that the mice rarely slept in the houses and chewed the chew toys initially with usage dropping off after a few days. Mice given nesting materials tended to stay in their nest. Many were found asleep at the daily clinical observation time and were easier to handle. Based on the results of this preference test, approved nesting materials will become the primary standard of our mouse enrichment program.

#### **P71 Choice as an Influence on Rates of Enrichment Manipulation in Rhesus Macaques (*Macaca mulatta*)**

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Manipulable objects, such as commercially available pet toys, have been found to reduce or eliminate abnormal behavioral patterns in laboratory macaques. Experience with provision of many kinds of pet toys to laboratory macaques suggested that individual preferences for objects might influence object manipulation rates. Here we report a study involving 16 rhesus macaques, randomly assigned into 2 study groups of 2 males and 6 females at an AAALAC-accredited facility, trained to choose enrichment objects when presented with 2 different commercially available pet toys. Pretraining was done on weekdays for 3 wk by simultaneously offering 2 desirable food items and instructing each animal to choose one. Whichever item was selected first was given to the animal. During phase 1 (PH1) of this study, 2 pet toys were simultaneously presented to each animal with instruction to choose one. Individuals in group A received the selected toy, while individuals in group B received the toy not selected. During Phase 2 (PH2), the groups were reversed using 2 different pet toys. Individuals in group B received the toy that was selected, and individuals in group A received the toy not selected. Individuals in both groups were monitored for 3 min after the toy was given to record manipulation time during both phases. Each phase lasted 5 d with 1 sampling period performed on each day. It was anticipated that manipulation rates would be higher when individuals received the toy that was chosen; however, no statistically significant difference in manipulation time was noted between the 2 conditions (Chosen: mean = 649 s, SD = 187.44 s; Not Chosen: mean = 634 s, SD = 255.63; Wilcoxon test,  $P < 0.94$ ). As thought, time spent manipulating enrichment increased significantly in group B from PH1 to PH2 (Wilcoxon test,  $P < 0.008$ ); however, a significant increase was also noted in group A (Wilcoxon test,  $P < 0.016$ ). This may suggest that both toys used in PH2 were more desirable when compared to the toys used in PH1. It appears that choice does not influence manipulation time overall, as long as both items are desirable; although, individual differences are still present.

#### **P72 Use of Laminated Cards to Validate Weaning Technique: A Direct Application of the 3Rs**

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Weaning 21-d-old mice can be challenging, especially in genetically engineered strains that may be small for their age. After animal care personnel have received training on mouse weaning procedures they are required to demonstrate their proficiency as part of the validation process. Live animals are preferred for training, but they are not always available, thus limiting the flexibility in scheduling training. Development of alternatives to the use of live animals in training fulfills the goal of the 3Rs of animal research. The purpose of this project was to develop an alternative that would increase the flexibility in scheduling weaning validation and decrease the number of live animals required for training. Adult and weaning-age mice with various coat colors were photographed. The photographs were laminated and placed in empty cages for separation according to age and gender. Cage cards, breeding cards, and census sheets were also laminated to allow for repeated use. Marks made with a dry-erase marker or china pencil could be easily removed and the laminated materials disinfected between uses. A weaning check sheet was developed to allow the trainer to ensure that no important steps were skipped during the validation session. Some difficulty was encountered in determining the age and/or gender of the mice in the photographs. However, because this can occur when working with live mice, it provided an opportunity to determine how the trainee might handle the situation. Although this method has only recently been implemented, it has proven to be a realistic alternative to using live animals when assessing the weaning proficiency of animal care personnel.

#### **P73 Effects of Human Handling on Adult Laboratory Rabbit Behavior**

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Adult New Zealand white rabbits were handled on a routine basis to determine whether such treatment would make their behavior more compliant and less fearful during routine laboratory handling. After being handled over 3 wk, these rabbits were evaluated by novel personnel and compared to minimally handled controls. Evaluators scored the rabbits on a subjective scale for their relative resistance to being scruffed and removed from their cages, being transported to a treatment room, and their behavior at all stages of the exercise. Upon evaluation, rabbits that had received handling treatment were scored significantly less resistant than nontreated controls. During evaluation, behaviors that the rabbits displayed when they were approached in their cages and while being stroked and restrained on a treatment table were recorded for all rabbits and compared among the most docile and resistant subsets. These subsets displayed different behavior profiles throughout the exercise. This study illustrates the potential for human handling to reduce fear of humans, neophobia, or both in laboratory rabbits. Thus, reducing stress and improving rabbit compliance in laboratory procedures as well as improving animal welfare through exposure to novel stimuli.

#### **P74 Specific Pathogen-Free Sheep Monitoring and Biosecurity**

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Specific pathogen-free (SPF) animals have been produced for agricultural and biomedical purposes for over 50 y. Some species, such as swine, have well-established accrediting and regulatory agencies for SPF herds. SPF sheep flocks, however, lack such organizations to dictate testing strategies, biosecurity standards, and surveillance programs. To overcome this, the current composition of the SPF industry was reviewed to explore standards and various monitoring schemes that could be applied to SPF sheep programs, such as New England Ovis (NEO) in Strafford County, NH. Over 2 dozen enzootic and zoonotic sheep pathogens, including Q-Fever and contagious ecthyma (Orf), were investigated. Information from government agencies, academic institutions, and SPF vendors was gathered along with consultations with veterinarians and SPF operational managers for additional insight. Farm inspection criteria from the National SPF Swine Accrediting Agency were applied to SPF sheep management while testing parameters were based upon guidelines from government animal health organizations, such as the United States Department of Agriculture (USDA) and World Organization for Animal Health (OIE). The ultimate goal of this project is to provide the research animal industry with a working document that includes a list of sheep pathogens and subsequent monitoring programs for each pathogen as well as recommendations on biosecurity to help define, document, and more effectively maintain a claim of SPF status.

#### **P75 A Novel Technique for Identifying Mouse Genders in Neonates**

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Researchers often require gender-specific neonatal mice to fulfill the requirements of research projects. To maximize use of a litter of pups for both the researcher's needs and future breeding colony requirements, gender identification of new litters as early as day 0/1 is necessary. Rather than using the routine anogenital distance, the observation of a black dot midway between the anus and the prepuce, has been identified by technicians as an adjunct to the other methodology as early as day 0/1. In black mice, this black dot has been correlated with male mice. In this study, we proposed the cause of this gender-specific anatomic visual marker to be the development of scrotal tissue. To determine the accuracy for using this anatomic marker for gender identification, we looked for its presence in black, agouti, and albino furred pups. We used 100 black, albino, and agouti neonates at 0/1 to 10-d-old to assess both the success rate as well as the age at which the dot would potentially no longer be reliable. Further evaluation of 1 black, albino, and agouti litter of mice at 0/1, 7, and 14 d of age, comparing the presence of the black dot, to the anogenital distance, and post euthanasia direct visualization of sex organs methodologies yielded understanding of

the most dependable technique for gender identification for each coat color. In black pups at 0/1, 7, and 14 d of age, sex identification using the black dot methodology was in complete agreement with the sex determination by anogenital distance and post euthanasia sex organ examination. However, black dots were never identified in any of the white mice examined, though there was 100% agreement between the anogenital distance and direct visualization methodologies. Observation in agouti neonates is unknown at this time as collection of data is in progress.

#### **P76 Evaluating Occupational Exposures to Animal Allergens in a Rodent Facility**

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Exposure to animal allergens is increasingly recognized as a potential occupational health hazard for animal caretakers, supervisors, and researchers. Workers' exposures to animal allergens may be assessed through monitoring both area positional and breathing zone samples to evaluate airborne animal allergen concentrations in assigned tasks. Current literature suggests a working occupational exposure limit of 5 ng/m<sup>3</sup>, be set as a ceiling limit, for both mouse urinary proteins (MUP) and rat urinary proteins (RUP). A risk-based monitoring plan can be used to assess worker exposure to MUP and RUP during routine husbandry and facility operational activities. After monitoring, procedural operations are categorized to identify allergen exposure levels. Tasks involving open dumping of soiled bedding and tabletop cage changing typically produce the greatest level of exposure. The monitoring results are compared with the suggested exposure limit to determine the need for modifications to engineering controls, personal protective equipment (PPE), such as use of an N-95 respirator, and operating procedures. Reduction of exposure to animal allergens can be achieved through feasible engineering controls, administrative procedures, work practice controls, and PPE. This hierarchy of controls, combined with medical surveillance, plays a vital role in protecting the health and safety of employees.

#### **P77 Fleece Tubes as Treatment for Alopecia Associated with Overgrooming or Hair Plucking Behavior in Adult Female Rhesus Macaques (*Macaca mulatta*)**

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Overgrooming or hair plucking behavior, which can result in significant hair loss or alopecia, is viewed as a behavioral problem indicating diminished psychologic wellbeing. One study examined the use of paint rollers and grooming boards coated with food material in rhesus monkeys over a 6-wk period without significant results. In this study, we examined the effectiveness of fleece tubes and puzzle feeders in 16 individually housed adult female rhesus macaques (aged 5 to 9 y) with alopecia over a 16-wk treatment phase. The animals were randomized into 3 conditions and given either a fleece tube (FT;  $n = 6$ ), a commercially available puzzle feeder (PF;  $n = 6$ ), or no device (CL;  $n = 4$ ). Percentage hair loss was scored weekly for each monkey by 2 independent observers. Our analyses showed that by 12 wk, the FT group had significantly less percentage hair loss compared to CL (repeated measures ANOVA;  $P < 0.05$ ). No significant percentage hair loss was seen in the PF group compared to CL (repeated measures ANOVA;  $P < 0.05$ ). These results suggest that in females, the use of fleece tubes may be an effective means of treating alopecia related to hair plucking. Fleece tubes have been in use at our facility as part of our behavioral plan to treat alopecia secondary to overgrooming or hair plucking. This study is the first to quantify the effectiveness of this device in female rhesus monkeys.

#### **P78 Training Aids for Tumor Measurement**

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A challenging aspect for many laboratory animal training programs is the proper training of rodent users to detect, measure, and differentiate among some common subcutaneous masses such as tumors, hernias, and abscesses. Institutional policy dictates that the experimental endpoint is a mass size totaling 1.5 cm for a mouse and 2.5 cm for a rat, resulting from the largest

diameter of each mass. Rodent users must be taught how to differentiate tumors from hernias and abscesses based on their location, reducibility, and consistency. It was commonly observed that many rodent users were inaccurately measuring the smallest diameter of an irregular mass or using excessive compression, and therefore, recording smaller-than-accurate measurements. These inaccurate measurements confused researchers as to whether their animal's mass had truly reached the maximum allowable size limit set forth by the institution. Due to health issues associated with tumor growth, hernias, and abscesses, the use of live mouse models for evaluation and measurement of masses was considered inappropriate for training purposes. Therefore, an effective replacement training aid was developed for researchers and vivarium staff using 2 toy animals, a mouse and a rat. Small beads of varying size and consistency were sewn under the fabric skin of the training aids to simulate subcutaneous tumors, hernias, and abscesses. These simulated masses were measured and an answer key developed to ensure consistency of all subsequent training. Placement and consistency of the simulated masses mimicked common rodent health issues such as inguinal hernias and preputial abscesses. Rodent users were then trained using the modified training aids to help correct and clarify some commonly misinterpreted aspects of the institutional guidelines such as proper measuring techniques. The use of standardized, hands-on training aids ensured consistent training outcomes, thereby allowing rodent users to more correctly assess the rodent's health and better follow institutional guidelines.

#### **P79 Environmental Presence of Mouse Parvovirus at a Large Research University: A Potential Risk to Research Colony Animals**

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Mouse parvovirus is one of the most common and problematic infectious pathogens affecting research animal colonies. Sporadic outbreaks can occur at research universities with noncentralized rodent housing. Mouse parvovirus is resistant to environmental inactivation, and shedding of virus by wild rodent populations into the environment and subsequent transmission by humans may be a source of these outbreaks. To assess the risk of human traffic as a vector for mouse parvovirus entry into mouse colonies we sought to determine the degree of environmental presence of mouse parvovirus on and around a large land grant research university. Environmental samples were collected from multiple locations, including loading docks near building entrances, animal transport vehicles, centrally located dairy and livestock facilities, elevators in animal housing buildings, and entrances to rodent housing rooms. Samples were sent to a diagnostic laboratory for PCR testing for mouse parvovirus. Virus was detected in samples collected in and around all agricultural facilities that were sampled, but not in other locations. These results indicate that mouse parvovirus can be present on the grounds of a research university at locations conducive for wild rodent populations such as in the vicinity of agricultural buildings. This environmental presence can be a source of infection if individuals walk past one of these areas and enter rodent housing facilities, serving as a vector for transmission of virus to otherwise naïve colonies. In conclusion, environmental presence of mouse parvovirus was demonstrated on the campus, including outside of centralized high traffic areas. In order to reduce risk of mouse parvovirus entry into animal colonies at large institutions where a wide spectrum of research is conducted, environmental sources of virus must be considered. This result reinforces the need for appropriate personal protective equipment requirements and effective standard operating procedures designed to minimize contamination of research rodents by human vectors.

#### **P80 Our Experiences with Anole Maintenance**

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Initiating an anole study in our facility was an interesting and unique experience. Upon assessing the needs of the anoles (*Anolis carolinensis*), we wanted to maintain the best environment for these lizards to thrive in, as well as facilitate sanitation. The study required housing roughly 40 lizards. We housed the lizards in rat cages with microisolation lids with the filter paper replaced with wire mesh. We started the design with a basic plan that had been used at another university from where this study had been transferred. Cages included a few basic amenities such as a stick and proper substrate.

For a water source, we would mist the cages daily. Lighting needed to be UVB. The food source, crickets, would also be dusted with vitamins and minerals created specifically for reptile needs. After reviewing some of the SOPs used by the previous institution, we noticed a few things that could be changed to make things both less stressful for the lizards and easier for us as keepers. Most importantly, we evaluated the substrate in the previous research; most investigators had used either a fake grassy substance or a cloth type material. We decided to try cypress mulch, which was very inexpensive and provided an appropriate absorbency. Also, it enhanced environmental enrichment and provided us with the ability to spot change. This allowed us to reduce the amount of time opening the lizards' cages and the frequency of full change outs. We were able to develop a plan using the best methods from the previous institution and incorporating new ideas that better fit our facility. Successful housing of a species less commonly used in research can be achieved with a combination of previous experience or published recommendations, ingenuity, and enthusiasm for caring for an interesting and challenging species like the green anoles.

#### **P81 Acclimation and Positive Human Interaction as Methods to Facilitate Handling and Reduce Stress in Captive Cynomolgus Monkeys (*Macaca fascicularis*)**

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Macaques are often used in biomedical research to discover and develop new treatments for human diseases. A unique challenge to managing macaques within a research facility is to provide an enriched environment that includes positive interactions with humans. This enriched environment promotes psychologic wellbeing of the animals and prevents abnormal behavior such as overgrooming, self injurious behavior, stereotypical movements, and increased aggression, or timidity. We investigated whether acclimation combined with positive human interaction would facilitate handling and restraint while reducing distress in a group of 8 female cynomolgus macaques. Prior to study, trained staff evaluated animals for their cooperation during the restraint chairing process and assigned baseline quantitative scores using a created grading scale. Extreme aggression/nervousness and lack of cooperation was scored a 1, whereas a 5 denoted no resistance to the restraint process while remaining calm throughout the session. Trained technologists also evaluated the animals for alopecia, using an objective 5 point alopecia scoring scale, 1 indicating within normal limits and 5 indicating completely bald. In this study, each animal was transferred from its cage to a standard NHP restraint chair via pole and collar and back to its home cage 3 times weekly using our established protocol of positive human interactions including positive reinforcement training, and desensitization. The animals were individually assessed during each session and scored based upon their cooperation during the transfer process. Cynos were closely examined once weekly and scored for hair loss. Preliminary results found a mean score of 2.125 for prestudy alopecia and 1.17 after 5 wk of intensive training. Mean scores for ease of handling increased from 3.25 prior to study to 4.125 after 5 wk. Final results after 12 wk of study will be available July 2009. To date, our study found that positive human interactions acclimated these 8 female cynomolgus macaques to standard handling and restraint techniques while reducing signs of distress.

#### **P82 Microbiologic Quality of Commercial Brands of Animal Contact Corncob Bedding: Potential of Sanitized Corncob Particles for Reducing Sterilization Costs**

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Costs associated with the use of animal bedding contribute to a large percentage of the operating expenses in maintaining a laboratory animal facility. Included in these expenses are the energy costs of physical interventions to sterilize bedding, including corncob particles that carry relatively high bacterial and fungal populations. The recent commercial availability of competitively priced, sanitized corncob bedding may substantially reduce energy costs due to decreased levels of antimicrobial treatments required to achieve sterility. Accordingly, a study was conducted to determine the microbial quality of 8 commercial brands of corncob bedding (including a

sanitized product) before and after autoclaving or electron beam irradiation and to evaluate the extent of these antimicrobial treatments required for sterilization. Numbers of viable microorganisms in homogenates of 25-g samples of bedding were determined by serial dilution (1:10) and plating on appropriate agar media. Three independent replications of each experiment were conducted, and duplicate samples were analyzed per treatment within each replication. Before autoclaving or irradiation, populations (log CFU/g) in nonsanitized corn cob particles ranged from 4.40 to 6.16 (aerobic plate count; APC), 2.05 to 4.23 (Enterobacteriaceae), 4.06 to 5.79 (aerobic mesophilic spores; AMS) and 2.08 to 3.93 (yeast and mold). In samples of sanitized particles the initial APC and numbers of AMS ranged from 1.44 to 2.84 and 1.48 to 2.76, respectively, whereas Enterobacteriaceae and yeast and mold were not detected (< 10 CFU/g). Nonsanitized particles required longer autoclaving times (60 min) or higher doses of irradiation (17.5 to 20 kGy) to achieve sterility. In contrast, sterilization of sanitized particles was achieved by autoclaving (250 °F, 15 PSI) for 30 min or with irradiation at 7.5 kGy. Based on these results the use of sanitized corn cob particles has good potential for substantially reducing costs associated with sterilization of these bedding materials for laboratory animals.

### **P83 Environmental Quality Assurance for Research Animals**

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Maintaining the research animals' environment within acceptable standards is a federal mandate. At the University of Florida, this is a collaborative task among the animal care services' husbandry, veterinary, and diagnostic laboratory staff. All animal housing units are sanitized monthly and tested with contact agar slides or luciferase-ATP test system. Representative water samples from different sources (ventilated rack, water filling station, and tap water) are tested monthly for bacteria, and additional testing package, which includes chemicals and pesticides, is done for newly opened facilities, facilities housing GLP studies, or rooms with persistent health concerns. SOP-defined number of items (cages, accessories, racks, food bowls, and enrichment toys) is tested weekly for ATP/bacteria as they come out of the tunnel/rack washers. All items going into the rodent barrier areas and coming out of the infectious disease suites are autoclaved, and microbial quality is assured with the help of biologic indicators. Infectious disease suites or rooms where excluded pathogens have been detected are decontaminated with vaporized hydrogen peroxide and validated with appropriate biologic indicators. Sample collection for these various tests is done mainly by trained husbandry personnel while test reading and results interpretation are performed by the diagnostic laboratory staff. All tests and test results are entered in the shared database, and a report is generated monthly to keep staff informed on the number and passing percentage of tested rooms, water samples, autoclaves, and washed items per facility. Aside from documenting the quality of environment provided to the different laboratory animals, the monthly report also serves as reference for discussing ways to improve the standards of care provided in the different facilities. This routine environmental monitoring practice contributes to the low to null incidence of common pathogens that are known to persist in the environment, specifically mouse parvovirus.

### **P84 Genetic SNP Test Establishes Country of Origin for Rhesus and Cynomolgus Macaques**

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Behavioral and physiologic differences between Indian-origin and Chinese-origin rhesus macaques have highlighted the critical importance of using purebred, single-origin animals in many research studies. Similarly, the genetic and physiologic variation that distinguishes cynomolgus macaques derived from Indonesia, Mauritius, Indochina, and the Philippines can affect the outcome of infectious disease as well as behavioral studies. Thus, there is a growing demand for ancestry testing of rhesus and cynomolgus macaques to ensure the consistent use of macaque populations in research studies and within breeding colonies. Towards this goal, we have sequenced the genomes of 24 rhesus macaques and 50 cynomolgus macaques derived from India, China, SE Asia, Indochina, or Mauritius to identify single nucleotide polymorphisms (SNPs) that are unique to each macaque sub-population. Combined in a high throughput SNP array format, the identified

ancestral informative markers (AIMs) distinguish rhesus and cynomolgus macaque origin, and detect hybrid rhesus macaques with as little as 25% mixed background. Using this assay, we have found that 6% to 8% of rhesus macaques, purchased over 10 y from 6 different rhesus macaque suppliers, were misclassified as to origin. Among 4 purchased cynomolgus macaque cohorts, we found little intra-cohort variation; however, 1 of the 4 groups was misclassified as to ancestral background. Thus the macaque ancestral SNP assay has become a valuable tool for screening and validating acquired rhesus and cynomolgus macaques, minimizing the risk of incorporating misclassified animals into breeding and research programs.

### **P85 Advancements in Recruiting Animal Care Staff**

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Recruitment of animal care staff into laboratory vivarium staff is reliant on targeting the correct audience. Historically, difficulties have been experienced in attracting high-quality recruits, such as those who have attended college and university graduates, into the animal technology industry. Some reasons for this are the lack of information and understanding about the role, no formal education on the subject of animal care in the research environment, and, sometimes, negative perceptions by the public and media. By developing a strategy to directly influence information given within animal care courses, or amending the course curriculum to include lab animal sciences, both the students and lecturers have received relevant information and, ultimately, another choice within the animal care industries. We outline the positive benefits seen by forming close relationships with local colleges and universities, and how, through providing further education on the subject, we have broken down some of the barriers and perceptions that may have caused problems in the past, resulting in a high number of quality applicants and an increase in graduate intake of between 25% to 50% for vacancies.

### **P86 Mouse Ear Tattoo: A Quick and Easy Alternative to Ear Punches and Tags**

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Some of the more common methods for individual mouse identification include tattooing of the tail or toes, ear punches, and ear tags. Each method has its advantages and drawbacks, so an alternative method was explored. The micro tattoo system is designed to use a standard hypodermic needle which is reinked in a well on the opposing side and is commonly used for tattooing the toes of mice. A study was performed to see if this method could be used to tattoo the ears of individual mice using a set pattern for numbering. The micro tattoo equipment, nontoxic ink, and a 25G needle, were used to tattoo small dots in a set pattern on the animal ears, giving each a unique number. The animal was gently restrained and the ear punctured the required number of times, excess ink was wiped away and the animals returned to their cages. The ability to quickly identify individual animals and the longevity of the tattoos were monitored over the course of multiple studies. The end result provided an easy method for tattooing and allowed for quick identification of any individual animal. The animals showed no adverse effect and the tattoo pattern has remained readable for 2 y. A standard identification chart that is used for toe tattooing can be modified or one created specifically by the user. A cage of up to 10 mice can be quickly tattooed, allowing for rapid visual identification without handling the animals. The process has been repeated using hundreds of animals and has produced excellent and reliable results with very little effort needed to identify the individual animals.

### **P87 Improvement of Canine Gavage through Behavioral Refinement**

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Purpose-bred laboratory beagles are a well accepted in vivo model for pharmacokinetic experiments evaluating drug absorption, distribution, metabolism, and elimination. Oral gavage is a common method for dose administration in the canine model. The traditional method for oral gavage of dogs involves the use of a bite bar with manual control of the mouth



while advancing a feeding tube into the stomach. The dog is restrained on a table and held close to the handler. The dose substance is subsequently administered through the feeding tube. As this method has been used, dogs are often uncooperative and struggle through the process. Apparent aversion to the restraint and placement of the bite bar creates a situation of injury risk to the restrainer, doser, and the animal. A novel technique of restraint was adopted and used during canine oral gavage that improved this situation and decreased the amount of struggling involved with oral gavage. Using the theory of canine subordinate behavioral response to a scruff hold, this novel restraint involves a scruff hold of the animal, combined with support of the rear legs. Other parts of the dog (head, front legs, and muzzle) can be restrained at the same time if needed. This improved restraint technique, proved advantageous for the animals and the handlers. The canines accepted this methodology quite well, presumably due to their innate response to this as a natural form of restraint. The subsequent decrease in uncooperative behavior enabled the technicians to work more routinely with the canines without feeling they were being forced into a negative situation. This technique eliminated the need for a bite bar, even when the feeding tube is passed. After adopting this technique, we have observed significantly lower instances of struggling during canine gavage, leading us to believe that this new method is a positive animal welfare benefit.

#### **P88 Development of a Standard Operating Procedure for Warming Hypothermic Mice Due to Flooded Caging**

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Leakage from the water valves on rodent automatic water systems can cause flooding and animals to become hypothermic if not found early. Heat lamps and microwavable heating pads have been used to dry and warm wet mice but none of these methods have been critically evaluated for effectiveness. The purpose of this study was to determine the optimal timing and distance for placement when using a heat lamp to warm mouse cages after flooding. The core temperature of a normal mouse is between 96.6 and 99.7 °F. The objective was to warm the cage quickly to ≤100 °F and then determine how long the cage could maintain this temperature once returned to the ventilated rack. An empty microisolation cage containing 1 cup of corncob bedding with the lid on and food in the hopper was placed 6, 12, or 18 in. from a standard heat lamp with a 250-watt bulb. The temperature inside the cage was measured with a data logger every minute for 90 to 280 min. Temperatures were also measured at 6 in. from the heat source for 6 h to determine maximal temperatures attained. Measurements were performed in triplicate under a class II biosafety cabinet with the blower turned on. At 6 in. from the heat lamp, it took 35 ± 3 min, at 12 in. it took 74 ± 8 min and at 18 in. it took 234 ± 22 min to reach 99.8 ± 0.1 °F. Humidity within the cage was maintained at 29.7 ± 0.2 for all experiments. Cages heated for 6 h reached a maximal temperature of 122 °F by 180 min. Cages heated at a distance of 6 in. to target temperature and returned to the ventilated rack maintained elevated temperatures for over 60 min. In conclusion, mice from flooded cages can be safely warmed by placement in a dry cage 6 in. from a heat lamp for 35 min and then returning to the ventilated rack. Cages should not be left unattended as internal cage temperatures can reach limits detrimental to mice. This provides a rapid, reliable, and inexpensive method for maintaining mouse health and welfare in the face of unexpected cage flooding.

#### **P89 Alopecia Pattern and Location Correlate with Hand Preference in Recently Relocated Adult Male Squirrel Monkeys: A Case Study**

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During quarantine, 8 of 12 adult male squirrel monkeys (*Saimiri boliviensis*) presented with a similar alopecia pattern/location. Monkeys exhibited mild unilateral or bilateral alopecia on the dorsal forearms. The alopecia (AL) was not associated with dermatitis or other health concerns and excessive grooming and/or scratching was not observed. The alopecia was noted between days 14 to 21 of quarantine. This specific hair loss pattern/location in macaque species housed at our AAALAC-accredited facility has been attributed to mechanical alopecia (that is, hair loss due to rubbing on the enclosure). Cases in macaques usually present as chronic, mild

hair loss without dermatitis. Due to the suspicion of mechanical alopecia, we investigated whether the pattern/location of alopecia correlated with hand preference (that is, LT hand preference = LT forearm AL, RT hand preference = RT forearm AL, no hand preference = both forearm AL). Hand preference was determined using a simple food reaching task ( $n = 30$  reaches per animal). A high commodity food item was held at the feed opening. The preferred manner for acquiring food items for all animals was through the smaller cage bar openings and not the feed opening. This resulted in contact between the animal's dorsal forearm and the cage bars. The alopecia pattern/location was positively correlated with hand preference (5 of 8 animals, 62.3%). More specifically we found that all cases of unilateral alopecia positively correlated with hand preference, while only half (2 of 4) of the animals that exhibited bilateral alopecia were scored as both bilateral alopecia and no hand preference. We concluded that the hair loss correlated with hand preference. All alopecia resolved within 6 wk. We suspect the etiology of the alopecia pattern/location to be due to a combination of factors including mechanical rubbing on cage, hand preference, and, potentially, relocation stress.

#### **P90 Promoting Animal Research and the 3Rs within the Community**

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Pfizer Global Research and Development has a long running school speaker scheme, in which trained colleagues visit schools, colleges, universities, and groups in East Kent to present on the use of animals in medical research. Regularly, the recipients had previously received talks or material from antivivisection groups, so our presentation helped students form their own educated and informed opinions on the subject. The research investigated the beliefs and opinions of young adults by ascertaining how much impact receiving a Pfizer school speaker presentation had, and assessed whether the talk influenced the opinion of the recipients. Opinion change was measured by the use of questionnaires provided to the recipients before and after the presentation. Data from the questionnaires was then statistically analyzed to gauge opinion change. Other factors such as opinions about the use of animals in the meat and farming industries, cosmetic testing, primates in research, zoos, pets, circus animals, and some ethical opinions were also examined. Interestingly, the results showed a positive opinion change towards the use of animals in medical research after the presentation, demonstrating what an important role promoting our work and education play in the understanding and acceptance of research. This is an important project, seeing as the audience is the next generation of adults. Providing education on the use of animals in research will shape the future, hopefully improving the current climate of the animal rights movement.

#### **P91 Rearing of Chicks in Germ-Free Isolators**

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Germ-free chickens are usually derived by prolonged immersion of late gestation specific pathogen-free (SPF) eggs in mercuric chloride. This procedure may result in decreased viability of embryos and chicks. The goal of this project was to determine if a less harsh sterilization procedure effectively prevents bacterial colonization while preserving embryo viability. Prior to entering the eggs into sterile soft-sided bubble isolators, the receiving isolators were supplied with a digital hygrometer/thermometer, autoclaved grit, 5065 chick chow, and sterile caging, including a gravity fed water dish and bedding. To maintain a 98 °F temperature, three 150-watt heat lamps were placed 5 in. away from the outer surface of the isolators. Inside the isolators, a linen towel soaked in sterile water provided a humidity range from 35% to 55%. On day 19 of a 21 d gestation period, 25 eggs were introduced into the sterile isolators. Under a Class II biohazard safety cabinet, the eggs were dipped aseptically in a 10% bleach solution for 7 to 10 s, followed by two 3- to 5-s rinse dips in sterile water. The eggs were immediately enclosed in a 5 in. × 12 in. polypropylene box with a solid microisolation top and placed in the receiving germ free bubble isolator port. The port was sterilized with a germicidal compound and given a 20-min contact period. All steps were completed within 1 h. On gestation day 21, 4 eggs hatched. On gestation day 22, 10 more hatched, followed by 7 the next day. Two eggs did not hatch and 2 chicks died during the hatching process. All chicks, fecal matter, and isolators remained bacteriologically sterile. The outcome of this project was

to demonstrate an ability to generate and maintain germ-free chickens with a high hatch rate. In addition, we were able to maintain constant temperature and humidity levels safe for the animals and the soft-sided bubble isolators. The method of sterilizing the eggs with bleach is safer than the use of a more traditional 2% mercuric chloride solution.

### **P92 A New System for Experimentally Induced Tumor Monitoring and Reporting**

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Animal models of cancer frequently require animals to experience tumor growth and/or metastasis. The unpredictable nature of cancerous cells complicates achieving the ultimate goal of balancing animal welfare and preserving scientific data. It is this unpredictable nature which makes setting universal endpoints for animals on protocols involving experimentally induced tumors challenging. We recently developed a new system for experimentally induced tumor monitoring and reporting. This tumor monitoring system was developed with the following goals: to assure welfare of animals on experimentally induced tumor protocols, to assure compliance with protocol specific endpoints, and to reduce the loss of scientific data due to unusual rate of tumor growth. The new system includes the principal investigator, husbandry, veterinary, and the University Committee on Use and Care of Animals (UCUCA—IACUC) compliance staff working as a team to accomplish these goals. Experimentally induced tumor models are split into 2 categories, animals in which the expected phenotype includes tumor growth and animals which are implanted with tumor cells. Cage cards are flagged for easy identification at weaning (for animals expected to grow tumors) or at implantation of tumor cells. A tumor monitoring sheet corresponding to the flagged cage cards is filled out by the laboratory staff utilizing unique observation codes for protocol specific endpoints. The husbandry staff is trained to report tumors at set universal time points to the veterinary staff. In addition, the husbandry staff is trained to check the monitoring sheets for completeness once a week. These monitoring sheets remain in the room available to the laboratory, husbandry, and veterinary staff or the UCUCA. This system was piloted for 3 mo with positive feedback in a small population and is currently on track to be used university-wide.

### **P93 Equipment and Cage Rack Modifications for Rodent Infusion Studies**

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Continuous infusion studies are commonly performed in preclinical safety studies to support clinical development. The reintroduction of the rat infusion study type at our facility required examination of current equipment and caging in an effort to design the most functional and efficient system. Cage sizes and types, ease of animal and equipment access, electrical requirements, tethering, and infusion pump options were all evaluated. The selected design was a luer locking system attached to a harnessed rat in standard shoebox caging with an automatic watering system allowing full access to the animal and the pump. This system ensures dosing integrity (accurate exposure necessary for safety studies) and, from an animal welfare perspective, it is an improvement over previous procedures.

### **P94 Prospective and Retrospective Analyses of Cage Flooding**

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During the course of routine animal husbandry, water bottle or automatic watering system malfunctions that result in cage flooding can be a source of significant morbidity and mortality in mice used in biomedical research. Preliminary studies indicated that up to 50% of adult mice and a greater percentage of neonatal mice in flooded cages are found dead or with some degree of morbidity. Approximately 25% of mice that survive cage flooding present moribund or dead within 1 to 3 d following exposure to wet bedding. A retrospective study on cage flood incident reports was executed to detect any trends and predisposing factors leading to morbidity and mortality following cage floods. Among other factors, variables such as investigator, animal care technician, animal strain, and room number were analyzed for

statistical significance. None of these was significantly related to incidence. Additionally, an overall cage flood incidence of 0.05% was derived. Animal wetness score versus behavior score, animal condition score versus clinical follow up, and animal behavior score versus clinical follow up were not statistically related. Further, behavior score, degree of cage wetness, and treatment follow up did not correlate with mortality outcome or align with causation. The primary treatment following a flood was to provide a clean cage. Institution of other treatments, such as use of heating pads and towel drying, was initiated in order to decrease post flooding mortality.

### **P95 A Critical Evaluation of the Efficacy and Limitations of ATP-Based Microbial Monitoring Systems**

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Monitoring of sanitation is an essential component of laboratory animal facilities. Typically, monitoring was done with solid surface agar contact plates. A significant limitation to this approach is that it does not detect all unwanted microbes but only cultivatable aerobic microbes. Our purpose was to rigorously examine an ATP-based monitoring system's ability to detect a variety of microbes (cultivable and noncultivable) and organic contaminants, and to compare the efficacy of the system to traditional methods of bacterial colony forming units (CFU) detection. Ten  $\mu$ L serial dilutions of *Escherichia coli*, *Staphylococcus aureus*, *Toxocara canis* eggs, *Toxoplasma gondii* tachyzoites, epithelial cells, and rodent blood, urine, and feces were analyzed according to the manufacturer's recommendations. Detection of *E. coli* was fairly weak and the limit was  $10^4$  organisms. Sonication of *E. coli* prior to analysis significantly increased detection ( $P < 0.05$ ) indicating that bacterial lysis was incomplete in the detection system. Detection of *S. aureus* was significantly greater than *E. coli* ( $P < 0.05$ ), with a limit of detection of  $10^2$  with sonication not affecting results. Detection of eukaryotic cells (*T. canis*, *T. gondii*, red blood cells, and epithelial cells) was robust with limits of detection ranging from 2 *T. canis* eggs to 10 epithelial cells. Urine was weakly detected with a limit of detection of a 1:10 dilution compared to feces with a 105 dilution reflecting the relative cellularity of these substrates. Detection of all cells except epithelium had a strong linear correlation ranging from  $R^2 = 0.993$  to  $0.998$ . The data demonstrate that the ATP system sensitively detects pure cells and organic contaminants with a strong degree of linear predictability. The only limitation of the system is its ability to detect gram negative bacteria because of incomplete cell lysis. The ATP system could serve as a suitable replacement or an excellent adjunct to standard CFU analysis.

### **P96 Drinking Water Contamination**

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Rodent drinking water bottle contamination is a serious issue in the laboratory animal environment; contamination in treated water can signal a serious health issue. We employ individual water bottles for our animal cages instead of automatic watering devices. House water is treated with hydrochloric acid to achieve a pH of 2.5 and then sterilized. Despite these measures, growth in the water bottles was discovered in the majority of the animal rooms. This growth was similar to a clear jelly forming in the water within 24 h of treatment. After testing of sources, it was determined that the distilled (DI) water sources was contaminated, and the autoclaving process was not fully killing the bacteria. In addition to the DI source being contaminated, it was also discovered that the caretakers were not fully changing out water bottles for all cages, but instead topping off due to the shortage of daily sterile water. The contamination issue was corrected by installing a UV light source through which the water passes prior to entry into the automatic dispensing equipment. In addition, the facility began purchasing sterile DI water to mix to the hydrochloric acid. Management enforced a full change out of water bottles when the staff changed caging. Our goal is to develop a clean production area where the water coming from the UV light source will be able to go straight to the animal rooms, bypassing the autoclaving process.

### **P97 Tracking and Predicting Emerging Trends in Primate Use and Demand**

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The Primate Resource Referral Service is an NCRR/NIH-supported resource facilitating the sharing of nonhuman primates and primate-related resources by investigators and institutions both nationally and internationally. In many respects the Primate Resource Referral Service functions as a barometer of the primate research community at large, foretelling the emerging shifts in primate use and demand. Statistical analyses of the incoming data on primate availability and requests have been made in an effort to predict emerging trends and future demand for specific nonhuman primates. Specific analyses include bivariate correlations between variables and autocorrelation to show periodicity in requests for particular species. An understanding of emerging trends will enable the Primate Resource Referral Service to take a more proactive role in identifying potential supply concerns, hopefully forestalling future shortages like the critical rhesus macaque shortage that came to national attention in 2002.

#### **P98 A Cost Savings Approach to Sterilized Contact Bedding**

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Pfizer mandates that contact bedding be sterilized prior to use in its rodent caging systems. Historically, when smaller bags were used, the palletted bedding would be autoclaved in-house to ensure its sterility. However, when we converted to an automatic bulk bedding delivery/dispensing system, it created a dilemma on how to accomplish this task. During the evolution and original completion of the automatic dispensing system, it was determined that the large bulk bags containing bedding should be irradiated to ensure sterility. This bulk bag irradiation was done at an offsite facility by the supplier. The multistep process included numerous associated costs that in the end significantly increased the cost of the final product. We modified the standard bulk bag delivery/dispensing system to accept the product directly from the bulk bag instead of using a silo system. The question was asked if the bulk bedding bags could be effectively autoclaved and sterilized on site versus irradiated to save money. To make this determination, several questions needed to be answered. First the bedding vendor had to verify that the bulk bag could handle autoclaving and continue to contain the product without failure. After that determination was positively made, testing was completed on the bulk bags that verified there was complete sterilization even in the center of the bulk bag. After all questions were answered, it was decided that we would adopt this new program. This process change has been very successful and has provided a cost savings of at least US\$9,000 per quarter, in the first year alone. In summary, this exercise has shown that there are still opportunities, through innovation, that can save on operating costs without jeopardizing the quality of the final product and maintaining a high standard of animal care.

#### **P99 Disinfection Efficacy and Environmental Contamination with *Corynebacterium bovis***

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*Corynebacterium bovis* infection is often endemic in athymic nude mouse colonies at academic institutions. Bacterial control and elimination are challenging as depopulation and restricted colony access are often not options. We evaluated the efficacy of disinfection of *C. bovis* contaminated cages in a tunnel washer and bacterial environmental burden in an immunodeficient mouse holding room containing *C. bovis*-infected mice. Aerobic microbiologic isolation used Columbia-colistin-nalidixic acid with 5% sheep blood medium with identification of isolates by colorimetric biochemical testing. Complete cage set-ups housing *C. bovis*-infected athymic nude mice ( $n = 8$ ) were sanitized in a tunnel washer, without use of chemicals, providing a 180 °F water final rinse. Prior to sanitization, 100% of the cage set-ups were culture positive for *C. bovis* while after sanitization all cage components were culture negative, indicating that the sanitization method provides effective sterilization for this bacterium. Passive and active air sampling techniques conducted in an immunodeficient mouse holding room confirmed *C. bovis*

to be an airborne contaminant despite stringent standards of operation. Settle plates ( $n = 13$ ) were positioned throughout the holding room for 8-h periods on various days and an Andersen air sampler, which impacts 28.3 L/min of air onto an agar plate for 20 min, was used on 2 d ( $n = 8$ ). Fifty-four percent of the settle plates and 50% of the Andersen plates grew *C. bovis* with representative samples confirmed with 16S rRNA sequencing. No bacterial growth was observed in settle plates ( $n = 7$ ) placed inside autoclaved individually ventilated microisolation cages on various ventilated racks for 24-h periods. Contamination of settle plates ( $n = 2$ ) with *C. bovis* inside a Class II Type A2 biosafety cabinet during cage change procedures suggested a means of cross-contamination. Our findings indicate that *C. bovis* can be a pervasive environmental contaminant in infected mouse holding rooms.

#### **P100 The Biology, Breeding, Husbandry, and Diseases of the Captive Fat-Tailed Jird (*Pachyuromys duprasi natronensis*)**

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The Naval Medical Research Unit No. 3, Cairo, Egypt, has been working with fat-tailed jirds (FTJs), *Pachyuromys duprasi*, since 1996. The founding animals lived in the Wadi El Natroun area of the western margin of the Nile Delta and were captured by a government licensed hunter. Their home range includes North Africa from Algeria to Egypt. These rodents have a rounded body with long fluffy agouti-colored fur on the dorsum and white on the ventrum. They have a highly distinctive, blunt, sparsely haired, club-shaped tail which has recently been established as an effective site for the development of cutaneous leishmaniasis. Their adult body length is 148 mm to 183 mm and the tails range in length between 55 to 62 mm. In the wild their body weight averages between 22 to 44.6 g. We describe the biology, breeding, husbandry, and spontaneous diseases of our captive FTJ colony. Knowledge is based on nearly a decade of experience working with this unusual rodent species. This social species adapts extremely well to captivity and their unique anatomy, small size, fecundity, and docility collectively serve to make this species an attractive laboratory animal.

#### **P101 Prevailing over the Pitfalls: Planning a Facility Move**

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Four rodent breeding facilities, including approximately 90 individually ventilated cages (IVC) racks, were consolidated into a new, 15,000 ft<sup>2</sup> facility. Management, investigators, facility operators, and care staff considered a list of planning areas, including strategic reduction in colony size, transportation, cross-contamination, breeding depression, and HVAC/electrical overload. Staging moves over an 8-mo period provided valuable lessons. Trend analysis aided in providing communication to all parties from 6 mo prior to through 6 wk post relocation. A 6-mo planner for caretakers and investigative staff preparing for a vivarium transition was created for the institution. Defining critical gateways through changeover, in the form of a guidance document, proved to be essential in alleviating stressors of animals, investigators, and care staff navigating through a facility move.

#### **P102 Revamping the Rollout of the Standard Operating Procedures**

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In the past, standard operating procedures (SOPs) at our facility were drafted based on the input of the managers and veterinarians. These drafts were then agreed upon by the experts in that corresponding area and then followed a process that included review by a multidepartmental SOP committee before being approved and effective. Once effective, the designated SOP trainer for each area reviewed each new SOP with the animal resource staff. The web-based document repository that housed the SOPs had to be manually checked for new effective procedures. SOPs being composed by management led to confusion with terminology, redundancies, and artifacts. Having to manually check for new SOPs and updates to SOPs resulted in training lags. The problem was noticed by animal resource managers after concerns were brought forward from the resource technicians. Based on



the aforementioned concern, resource managers assembled a committee of resource technicians to be included in the review of the SOPs draft. A new web-based document management system was also employed to send out approval and effective notifications. Now that resource technicians have a part in the review process, the SOPs are more understandable, less repetitive, and are being continuously updated. The experts assigned to the corresponding SOP still approve the draft before it continues through the process. The resource technicians assigned to the review committee are also the designated SOP trainers in their corresponding buildings. Involving the technicians in this process, with the addition of the SOPs email notifications, has allowed SOPs training to occur more frequently and facilitate maintenance of current training records. Furthermore, questions and concerns are being addressed earlier.

### **P103 Experimental Endpoints: A Review**

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Establishing humane experimental endpoints is an integral part of an animal research study. These are predetermined criteria used to judge when research animals should be euthanized to minimize their distress or suffering. It is mandated by federal law and regulations and strongly advocated by organizations like AAALAC International. There are different facets when establishing humane endpoints. These include the roles played by various levels of the laboratory animal workforce, from the IACUC to the veterinarian and animal caretakers. There are also considerations in the implementation of such guidelines, and these may include scientific rationale as provided by the principal investigator. Meanwhile, common research manipulations like tumor burdens and veterinary cases like body weight loss are also common guidelines, although considerably varying endpoints are implemented. Finally, carrying out pilot studies in some cases (for example, developing new animal models), establishing score sheets catered to particular studies (for example, experimental autoimmune encephalomyelitis), and performing postapproval monitoring are important.

### **P104 Ventilated Caging Systems Can Induce Hypoxic Preconditioning**

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Comparisons of the microenvironment have been studied in individually ventilated cages versus microisolation cages. However, there is a paucity of information on microenvironmental differences between individually ventilated cages and wirebar top (shoobox cages) especially pertaining to oxygen concentration. We have shown that shoobox cage-raised 8 to 12-wk-old male C57BL/6J mice exposed to acute hypoxia (8% oxygen/92% nitrogen, for 2 h) become sick as evidenced by social withdrawal. Importantly, these mice fully recover from this sickness symptom within 6 to 8 h after return to atmospheric oxygen concentrations. When mice are raised in a ventilated cage system, recovery from acute hypoxia was shortened to 2 to 3 h. Examination of ventilated cage oxygen concentrations revealed 21% oxygen when no mice were housed in the cages examined. However, when mice were housed 4 to a cage in ventilated cages for 1 wk ( $n = 6$  cages  $\times$  4 mice; 24 mice total), oxygen concentration dropped to 20.5%. Relative humidity increased from 33% to 47% in ventilated cages with animals and remained unchanged in shoobox cages with animals (33%). These data indicate that ventilated caging systems have up to a 0.5% reduction in ambient oxygen compared to shoobox cages, which can induce hypoxic preconditioning.

### **P105 Use of Microfiber Mops to Clean Floors of Aviaries Housing Zebra Finches (*Taeniopygia guttata*)**

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Free flight aviaries (92 cm  $\times$  152 cm  $\times$  183 cm) are used to house up to 75 adult zebra finches (*Taeniopygia guttata*). The aviaries rest on epoxy-painted cement floors. During weekly sanitation of the room, the aviaries (with birds) were moved around the room allowing access to soiled floors. The

floors were cleaned with mechanical scrubbing and mopped with dilute chlorhexidine solution using a standard string mop. Routine sanitation monitoring program repeatedly demonstrated inadequacies with the existing floor cleaning method, as evidenced by colonies on contact agar plates that were too numerous to count following cleaning. Alternate approaches to improving the cleaning program presented challenges as the birds must remain in the room. Chemical agents with harsh fumes and those that may leave a hazardous residue were excluded. To minimize stress to birds and be consistent with research objectives, loud noises, such as power washers, were avoided. Acknowledging sanitation trends within human hospitals, a pilot project involving the cleaning of aviary floors with microfiber mops was instituted. Microfiber mop heads are composed of nylon fibers that are approximately 1/16 the diameter of a human hair. The dense fiber construction allows them to absorb up to 6 times their weight in liquid. The fibers have a positive charge that attracts negatively charged dust particles, and the fibers readily penetrate any irregularities in the hard surface. This allows for the mechanical removal of microbial contaminants. Prior to the introduction of the microfiber cleaning technique, contact agar samples collected after cleaning demonstrated plates with microbial colonies that were too numerous to count. The microfiber mops were used with dilute chlorhexidine weekly for 4 wk. At the end of this 28-d period, contact agar samples collected after cleaning revealed less than 10 colonies per plate. Furthermore, the husbandry staff reported ergonomic benefits including ease of use. We have incorporated microfiber cleaning into our standard procedures for sanitation of zebra finch aviaries. Subsequent routine sanitation monitoring has demonstrated the continued effectiveness of the technique.

### **P106 Surgical Support Procedures at NIAAA Facilities**

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Management of postoperative care procedures for research animals is a concern in the laboratory animal facilities. The transfer of information from animal care staff to researchers and veterinarians is a procedure that requires attention to detail. Our surgical support process is streamlined to allow accurate information flow. Both sites use a digital database that tracks all pertinent information for each surgical case. Rodents that are scheduled for surgery are tracked individually. Every time an animal receives clinical support or the facility staff conducts technical procedures, including abnormal observations, the information is entered and, therefore, shared with researchers and veterinarians. The implementation of postoperative care begins with the researcher's call for surgical support, which is done via the technical services request form. Once this request is made, the technician creates and maintains the surgical record by updating the digital and paper tracking files (backup). The surgical record provides relevant information such as emergency contact information, treatment options, required analgesia, pre and postoperative body weights, supplements, and pain indicators. The investigators and technicians simply initial and record the time or appropriate scores (for example, pain and body condition) in the corresponding field. The preliminary form and number/initialing system for treatments has increased efficiency of postoperative procedures by simplifying the process for investigative staff. As mentioned previously, the data from each animal receiving surgery is entered into the program initially, and animal condition information is entered daily. Sharing of this information amongst the investigators, veterinarians, and laboratory animal technicians has proven effective. Providing up-to-the-minute information about the animals' changing conditions and completed treatments ensures that requested care is given to the animals, and the process that begins with a technical service request ends with the return to normal husbandry operations.

### **P107 Use of Enrichment to Reduce Feces Smearing in Rhesus Macaques**

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Feces smearing is a relatively common behavioral problem for rhesus macaques maintained in cages in biomedical facilities. There are few published studies examining potential treatments for this undesired behavior. Animals with this problem are often provided with some sort of object onto which they can redirect the behavior. In the current study, we examined the effects of 2 enrichment devices, a smear board (an example of the commonly used device, in this case a small plastic tray hung from the front of the cage) and

a porch (a small cage hung on the outside area of the home cage) on feces smearing. Eight adult rhesus macaques (*Macaca mulatta*) with a history of this behavior were subjects for this study. The monkeys were housed in single cages in rooms containing 16 to 44 conspecifics. We scored the amount of feces on the sides of the cage on a scale from 0 (no feces present) to 4 (majority of the cage covered). Observations were taken daily for 2 wk to establish a baseline score. We then gave half of the monkeys a smear board, which was covered with peanut butter 3 times/week. The other half received a porch, which was hung on the monkey's cage continuously. We scored the feces smearing daily for 2 additional weeks. Overall, the monkeys had significantly lower feces smear scores when either device was present compared to the baseline period (approximately 33% decrease; Wilcoxon  $Z = -2.5, P = 0.01$ ). Surprisingly, the average decrease was the same for both groups (Mann-Whitney  $U = 11, P = 0.39$ ). While the smear board allowed the monkeys to express the smearing behavior, the porch allowed the monkeys to watch others in their room. More work is needed to determine the long-term effects of these, and other, enrichment devices on feces smearing.

#### **P108 Designing and Managing Shared Behavioral Testing Suites in a Barrier Facility**

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Behavioral testing (BT) is a powerful tool for measuring behavioral phenotypes and genetic expression by evaluating reflexes, motor function, learning, memory, and sensory perception. BT suites offer diverse platforms to study cognitive neuroscience, but managing these can be very complex, especially as a shared resource. Ideal BT areas should be secured, environmentally controlled, easily decontaminated, and able to accommodate a variety of specialized equipment. To meet the needs of 33 research labs that share a barrier mouse facility, we designed dedicated, sound-attenuated testing suites consisting of 8 modifiable, 10 ft × 10 ft rooms arranged around a central anteroom. A proximal location to animal holding rooms was chosen to help mitigate stress due to transportation and minimize biosecurity risks outside of the facility. Each room contained a modifiable umbilical chase offering water, CO<sub>2</sub>, and air. Additional lines could be added without impacting ongoing studies. Entry doors were equipped with a keycard access and scan activated button operator that tempered noise and vibration. Environmental parameters were recorded by data loggers and rooms were outfitted with independent light timers to provide white or red-filtered for reverse light cycles. Planning and communication with end users was vital to successful management of the area. New BT users attended a requisite orientation that outlined suite capabilities, pathogen control, facility and researcher responsibilities, points of contact between labs, and storage space constraints. A web-based scheduler was employed to aid investigators in advanced planning with suite availability and reservation. By incorporating flexible room design, electronic reservations, and user orientation, we have successfully managed the BT suites with minimal oversight of trained facility staff. Opportunities encountered included supporting changing research direction, maximizing storage, management of equipment, and pathogen control. The customizable BT suite design together with an integrated management program allowed researchers to independently conduct behavioral testing with minimal delay or interference.

#### **P109 Alternate Canine Inhalation Restraint Model**

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The traditional restraint system used on canine inhalation studies consists of a hammock (sling) supported by a metal frame. This model requires a significant amount of training time for a 1 h exposure, approximately 3 to 4 wk. In addition, the traditional model offers little flexibility for canines of varying size. The proposed restraint system consists of a platform and a simple harness fastened to a lead. The new design allows the canine more freedom of movement within the boundaries of the platform. The new restraint system is intended to reduce training time, increase test system compliance, reduce stress, and improve overall animal welfare compared to the traditional model. A comparison between the traditional and the new design while the animal is connected to a mock inhalation system was examined, and we also investigated animal behavior, compliance, and stress using the new form of restraint. Behavior and compliance were sub-

jectively evaluated; stress was evaluated by monitoring cardiovascular and respiratory parameters that may impact the results of a standard inhalation toxicology study. During phase 1, 2 canines were introduced to the new restraint system. Appropriate harnesses and tethering points were chosen for the study. During evaluation, the animals were well behaved and more compliant than observed using the traditional model of restraint. Overall, stress of the animals decreased. Engineering modifications were made to the new system and implemented during phase 2, which is currently in progress. Four animals are being used on each restraint system (2 males and 2 females). In addition to respiratory measurements, blood pressure and heart rate are being recorded with a tail cuff as an additional measurement of stress. The animals are being dosed with saline via inhalation to view the effects under more appropriate study conditions. Results are pending.

#### **P110 The Southernmost Specific Pathogen-Free Mouse Facility of the Americas**

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The specific pathogen-free (SPF) animal facility of the Unit of Functional Genomics at Center of Scientific Studies (CECS) in Valdivia, Chile, was opened in late 2005. The design and operation of the barrier-generated environmental conditions in which mice are housed under rigorous sanitary and pathogen free standards are aided by professional and technical staff who follow standard operating procedures. A sentinel mice program allows the analysis of a complete panel of viruses, bacteria, and parasites every 4 mo by a world-renowned laboratory. In addition, microbiologic environmental monitoring is performed on water, air, surfaces, and materials. To meet high quality standards, this mouse facility purchases mice, food, bedding materials, supplies, equipment, spare parts, and others from internationally recognized sources. Within the sanitary barrier there is a laboratory in which transgenic mice are generated on a weekly basis to meet research projects from local investigators of CECS and also from other laboratories in Chile. Recently, we exported the first transgenic mouse model to the University of Miami, FL for a study on the genetic basis of kidney failure. The Functional Genomics Unit at CECS is also committed to the formation of human resources in the field of mouse molecular genetics and laboratory mouse science. Since its inauguration we have organized 3 international advanced courses devoted to the study and generation of genetically modified mice and related topics.

#### **P111 Survey of Several Pathogens in Chinese Zebrafish Research Facilities**

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Concerns about infectious diseases in fish used for research have risen with the dramatic increase in the application of fish as models in biomedical research. Few epidemiology studies are done about aquatic pathogens in Chinese zebrafish research facilities. In this study, we sampled 150 adult zebrafish by stratified random sample method in 5 research facilities in Shanghai. Twenty fish pathogens (epizootic haematopoietic necrosis virus, infectious haematopoietic necrosis virus, *Oncorhynchus masou* virus, spring viraemia of carp virus, viral haemorrhagic septicaemia virus, channel catfish virus, viral encephalopathy and retinopathy virus, infectious pancreatic necrosis virus, infectious salmon anaemia virus, epizootic ulcerative syndrome virus, *Renibacterium salmoninarum*, *Edwardsiella ictaluri*, *Piscirickettsia salmonis*, *Gyrodactylus salaricus*, red sea bream iridovirus, white sturgeon iridovirus, koi herpesvirus, *Pseudoloma neurophila*, *Mycobacterium* spp., and pathogenic *Aeromonas hydrophila*) were surveyed using RT-PCR or PCR according to pathogen RNA or DNA. The fish were euthanized by anesthesia overdose. The brain, gill, and internal organs were moved, excised, and homogenized, followed by the commercial kit protocol to collect both RNA and DNA solution from each sample. The RT-PCR tests were performed following instructions from the World Organization for Animal Health Manual of Diagnostic Tests for Aquatic Animals and relative reference papers; the PCR products were sequenced. Tested samples were found to be negative for all RT-PCR assays except the *Aeromonas hydrophila* 16SRNA, for which 5 fish samples tested positive. The sample submission information shows that all 5 fish were bought from the same aquarium vendor instead of coming from institutional breeding.

### **P112 Circadian Changes in Behavioral Thermoregulation and the Preferred Ambient Temperature in Two Species of Rats**

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The ambient temperature (Ta) at which lab rodents are housed has a profound impact on health and wellbeing. Current guidelines recommend housing rats at Ta of 18 to 26 °C, which is thought to be below the preferred Ta for this species. However, the only data to examine preferred Ta of rodents were conducted under chronic stress conditions, suggesting error in these measurements. Chronic cold stress has widespread effects that could adversely affect animal health and experimental outcome. We exposed unstressed Sprague-Dawley (SD) and Fisher 344 (F344) male rats to a thermal gradient (Ta range 15 to 40 °C) to determine their preferred Ta during the circadian cycle while measuring core temperature (Tc; °C) and motor activity (MA; m/h) using intraperitoneally implanted radiotelemetry devices. Ta, Tc, and MA responses in the gradient were compared to responses observed during housing at a constant Ta of 22.6 ± 0.1 (control). The preferred Ta of SD rats decreased from 25.7 ± 0.7 to 23.7 ± 0.3 from day to night ( $P < 0.05$ ) while the F344 rat's preferred Ta was 27.8 ± 0.3 (day) and 26.4 ± 0.5 (night) ( $P < 0.05$ ). The preferred Ta of each species was both at the warmer extreme of current guidelines and significantly greater than Ta of control group rats ( $P < 0.01$ ). Analysis of interspecies differences revealed that F344 rats preferred significantly higher Ta than SD rats. They also had a lower MA (SD: 9.5 ± 1.3 day and 33.1 ± 2.8 night, F344: 3.6 ± 0.8 day and 16.2 ± 2.8 night, interspecies  $P < 0.01$ ) but similar circadian Tc (SD: 37 ± 0.04 day and 37.6 ± 0.03 night, F344: 37 ± 0.02 day and 37.7 ± 0.05 night). These data suggest that F344 rats prefer warmer Ta, may not depend on behavioral thermoregulatory mechanisms as much as SD rats, and may instead prefer to alter metabolism to maintain Tc. The data also demonstrate that if given the opportunity to choose Ta, both SD and F344 rats prefer Ta above the current housing guidelines. These guidelines are likely inadequate and future revisions should consider both circadian changes and species differences. Research supported by Grants for Laboratory Animal Science (GLAS) and The National Science Foundation-Research Experiences for Undergraduates (NSF-REU)

### **P113 The Development and Implementation of an Environmental Monitoring Program**

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The development of the environmental monitoring program was designed based on the need to determine the ability to efficiently and effectively sanitize the animal care facilities, including caging equipment, work/room surfaces, doors, and floors. In our 5 animal care facilities, varying methods of cleaning and sanitation were observed. Due to lack of standard operating procedures, training, and methods, we were unable to evaluate the efficacy of the sanitation process. To accomplish this goal, monitoring equipment and standardized methods were instituted, testing areas established for base line data, and monitoring performed within the 5 facilities on a quarterly basis. It was determined that 2 sources of monitoring equipment would be used, adenosine triphosphate (ATP) sanitation monitoring system that measures relative light units (RLU) and D/E neutralizing agar with medium developed to neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals that measures colony forming units (CFU). Testing was performed in preestablished areas in the animal facility, such as caging equipment, work/room surfaces, doors, and floors, and as expected many different levels of facility sanitation was observed. Baseline data were reviewed and several hot spots were targeted as priorities. Acceptable microbial levels were established based on the facilities viral designation, traffic pattern and equipment usage. Standard operating procedures (SOPs) were written in a manner that defined chemical concentrations, cleaning and disinfection frequency, and specific methods used to accomplish these acceptable microbial counts for targeted hot spots. The implementation of SOP training sessions and proficiency certification followed. Upon completion of the first year, quarterly monitoring indicates that development of the environmental monitoring program has reduced microbial counts to within acceptable levels.

### **P114 Enriched Bedding: Saving Time, Money, and Reducing Variables**

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Advancements in rodent bedding manufacturing have resulted in the introduction of shredded paper strips as part of the bedding, thus providing substrates within the bedding material that promote the species typical behavior of nest building. We evaluated one of these preenriched bedding materials, comparing it to our traditional enrichment of compressed paper nesting squares as well as to sterilized, recycled paper strips. Using our sentinel colony (Rats: CD and mice: Swiss Webster, all housed on alpha cellulose bedding) we established how much of the original nesting material (cotton fiber sheets) was being gathered and if it was being used. Following 4 wk of baseline data collection we then introduced the enriched bedding (alpha cellulose bedding). We allowed the animals 14 d to acclimate to the new material and then performed focal observations once daily, conducted at varying times of the day. We evaluated the percentage of nesting material collected and whether or not it was being used. Utilization was determined by observation of an animal sitting in or on the nesting material. Using the same animals, we then transitioned the bedding to a nonenriched bedding, providing shredded, sterilized paper (recycled from our facility), and after a 14 d acclimation period, performed focal observations once daily. We again evaluated the percentage of nesting material collected and whether or not it was being used. We found that in 1 species (rats) the likelihood of the animal using the enrichment increased and that overall rodents in our facility used the enrichment material to perform species typical nest building behavior. Overall, the ease of using the preenriched bedding outweighed other options, both of which had higher hidden costs such as the costs of gas sterilizing the recycled paper strips and the cost to the research in the way of added variability.

### **P115 Indirect Spot Checking for Completion of Husbandry Duties**

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When managing a group of technicians, one must have faith that the information they document is accurate. During the initial training phase, direct oversight ensures the accuracy of recording daily tasks. The challenge for supervisors becomes follow-up to confirm that the tasks are continually and consistently performed at this level. We have developed a method to check for task completion that involves using removable stickers that are preprinted with specific questions relating to the animals in the cage. Examples included, "how many animals are in the cage?" and "what is the date of mating of these animals?" The stickers are then placed on the exterior of the cage in a subtle location. The person applying the sticker places a second sticker with the same question into a log book and records the date and location of the sticker, as well as the proper answer to the question. As the animal care technician performs their daily health observations, they should be viewing the entire cage to ensure that there are no problems. If they are performing their duties accurately, they will see the sticker on the cage and follow through by delivering the sticker to the supervisor and answering the question. The supervisor compares the answer to that in the log book and gives feedback accordingly. With this method, the sticker should be returned within 24 h. If it has not been returned in that time frame, the supervisor follows up with the technician who was responsible for the room on the day the tape was applied. This gives the supervisor the opportunity to work with the technician to determine how the training tool was missed, which leads to enhanced future job performance. We have been using similar methods for the past 5 y, until finally evolving to this more simplistic form during the past 2 y. The stickers are returned within 24 h 95% of the time. Since introducing this training tool, observations of unusual situations have improved dramatically. We feel that there is a correlation between this training tool and the enhanced attention to detail.

### **P116 Using a Spreadsheet to Record Animal Health Observations in a Decentralized Facility**

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We have, in addition to primate housing buildings, 3 separate facilities housing various small laboratory animals. Health observations are performed each morning for all species, and concerns are brought to the attention of the Clinical Medicine Unit. In the past this process was cumbersome as the clinical medicine technicians visited each of the 3 small laboratory animals unit (SLAU) buildings to evaluate paper records for reported health concerns. The SLAU housing areas are separate from the primate housing areas, and as there are few cases requiring clinical attention, the technicians spent time traveling to buildings where all animals had been reported as healthy for the day. In an effort to increase efficiency for the technicians, a new method of presenting health concerns was necessary. Using a spreadsheet program, an electronic daily observation sheet (DOS) was created via a workbook with separate spreadsheets for each animal room. The DOS was available over a secured network where any member of staff could have access to shared files. We designed a template to ensure that all data was consistently recorded. As a member of the SLAU found a health concern, they recorded the case. Cases would then be color coded using the 'fill' feature of the program to inform the technicians if a case had been treated by the SLAU staff. The technicians were then able to see all of the cases from their office, make comments for treatments and travel more efficiently to the facilities, including being able to plan room order approach based on specific pathogen-free, experimentally infected rooms, and quarantine. Technicians updated the DOS, again using color coding, to inform the SLAU technicians of information pertinent to the case. This electronic system resulted in significant increase in efficiency for technicians. As medical conditions resolved, the data was moved to an archive folder so as not to lose the information, which has had the added bonus of allowing us to search for old cases. Also, it is possible to compile information regarding health concerns pertinent to a specific researcher or to a specific health concern. Creating this system was easy and it is simple to use, and could be a useful tool for others that do not have electronic management software.

#### **P117 The Use of a Novel Dietary Gel Formulation for Hand-Rearing Guinea Pigs**

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A hand-rearing technique was developed to rederive adenovirus-free guinea pigs from a seropositive colony. Because seropositive animals tend not to shed adenovirus and guinea pigs are born precocious, natural parturition followed by rapid removal of piglets and hand-rearing was selected as an alternative to cesarean rederivation. This allowed sows to produce multiple litters, ultimately requiring fewer sows for rederivation. Females were radiographed weekly and parturition was estimated at 4 wk after fetal ossification. During the final estimated week of gestation, sows were monitored twice daily and palpated for detection of pubic symphysis dilation. Following parturition, litters were removed from the parental cage and room within 12 h. Piglets were weighed and syringe fed a canine milk replacement formula multiple times daily, with decreasing frequency and weaned over 7 to 10 d. Also, piglets were fed a replacement gel formulation, moist guinea pig chow, and a water bottle for 30 d. The gel consists of 2.5 cups of water, 4 tablespoons canine milk replacement formula, 4 tablespoons nutritionally complete herbivore recovery vitamin C enriched powdered food with high fiber timothy hay, and one 7 g envelope of unflavored gelatin. Ingredients are mixed into a large bowl and then poured into 2 tablespoon cups. The gel is placed in the refrigerator for at least 30 min. After 30 d, piglets were placed on an auto-water bottle and dry guinea pig chow. The use of the dietary gel facilitated early weaning from manual feeding of formula, resulting in a lower risk of aspiration. In addition, the gel allowed for a smaller frequency of formula feeding, eliminating the need for overnight feedings and additional staffing. On average, the piglets were weaned after 7 d of formula feeding cutting the average weaning time in half. Also, the gel contains fiber, which promotes gastrointestinal mobility and reduces intestinal dysbiosis. Rapid removal from a seropositive sow with hand-rearing of offspring can be an efficient and cost-effective alternative to cesarean rederivation and cross fostering. The methods described may also be successfully used in similar rederivation efforts or when parturition complications leave orphan piglets in a facility.

#### **P118 Performing Postapproval Monitoring of Animal Care and Use Protocols**

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The IACUC and Office of Animal Care Compliance (OACC) perform the semiannual inspections of laboratories and the animal care program. However, in some areas this oversight is neither frequent nor thorough enough to identify areas of noncompliance. The process does not engender the ability to provide immediate mentoring and training; thus, problems often recur if not managed in other ways. The USDA, OLAW, and AAALAC International expect institutions to implement sufficient oversight of animal programs to assure compliance. They often recommend programs like postapproval monitoring (PAM) as a method to improve oversight and compliance. Implementing PAMs will ultimately decrease the number of noncompliance issues and increase the effectiveness of approved IACUC protocols. It is important to note that areas of noncompliance that have gone initially undetected under our current oversight program will likely be identified. The OACC first developed standard operating procedures (SOPs) to be followed during the PAM process. We then developed, distributed, and implemented a PAM checklist that was used during the process to proactively communicate expectations, promote training and discussion, and to document outcomes. We performed 59 PAMs between January and December of 2008. There were 23 minor findings and no significant findings. A minor finding was defined as a deficiency that would not have a detrimental effect on animal welfare while a significant finding may have a detrimental effect on animal welfare. During and after PAMs, we offered training and/or recommendations as needed to refine animal care and use procedures. Since implementing our PAM program, there have been fewer findings on the semiannual inspections and on our regulatory agency findings.

#### **P119 An Alternative to Adhesive Tapes in Animal Facilities**

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Labeling of cages and items is critical to efficient management of animal facilities. Frequently, this is accomplished using adhesive tags or tapes. However, the use of adhesive materials has been discouraged by AAALAC site visitors and USDA inspectors because of the potential for bacterial growth in the adhesive residue that is often difficult to fully remove from the cage surfaces. The area in which we had the most pervasive use of tape was in our zebrafish facility. From the day the zebrafish facility was opened, there has been a quest to find a solution for labeling the fish tanks. Things tried include small card holders and a variety of tapes and labels. The card holders were easily bumped off the tanks, while the tape and labels were not easily removed. Over the years, the best solution was colored labeling tape; permanent markers could be used and the tape could be removed from 1 tank and then placed on another. However, the transfer of the tape left a sticky residue and eventually the tape would become worn. Old tape had the tendency to fall off of the tanks. After investigating and trying many different labels, a waterproof removable label with the properties of a vinyl cling material that can be run through a thermal printer was found. The label is large enough (1 in. × 3 in.) to allow for color-coding dots to be used with it. The label is easily removed from 1 tank and placed on another. As of yet, a limit to the number of times that the label can be transferred has not been established as no label has failed in a year, which is how long they have been in use. Since discovering this labeling system, we have found unlimited applications for it, both within the zebrafish areas and the rest of the animal facilities.

#### **P120 Ergonomic Issues Associated with Multi-Tiered Zebrafish Systems**

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As the designers of the zebrafish housing systems attempt to provide the most tanks within a set footprint, they tend to overlook the practical issues associated with high racking and overcrowding of tanks. As the rack height increases, personnel must use stepstools to reach the top 2 to 3 rows. Stepping on and off a stepstool in an area that is frequently wet is both a slip hazard and a repetitive motion injury hazard. When the tanks are scheduled to be changed,

the animal caretakers are moving up and down the stool all day, which has caused strain on the ankles and knees of the staff. To assist the caretakers, we have purchased a hydraulic lift that acts as a table for the tanks. We also purchased several wider, sturdier 3-step platforms to replace the traditional stepstools or small ladders. The surface area on the lift allows for several tanks to be prepared and changed without requiring the caretakers to step off of the platform. The number of times that they are required to move up and down the steps has been reduced by 75% to 90% on changing days, depending on the tank sizes. Since the purchase of the hydraulic platform, we have found many other ways to use it in the daily operation of the zebrafish facility.

#### **P121 Mouse Bucks: A Form of Laboratory Animal Technician Recognition and Acknowledgement**

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For the technicians taking care of research animals, it is important for them to operate at their best physically, mentally, and emotionally. The feeling of making a difference, having an impact, and being recognized can have a significant effect on the technician's psychologic state. The recognition committee at our institution designed and implemented a program called mouse bucks. A survey was taken and the majority of the animal technicians felt recognition was very important to them. Mouse bucks is a program in which managers, supervisors, and peers can recognize a job well done, whether it be helping a fellow technician or going above and beyond the call of duty. Mouse bucks put the power of recognition into everyone's hands. Mouse bucks can be traded in for any item on the list devised by the recognition committee. One mouse buck has the value of 1 dollar. It can be used alone or it can be saved up to purchase items of greater value. At the US\$1 level, items such as candy, chips, and chapstick are available. At the US\$5 level one can obtain a gift certificate of choice. Technicians can save their mouse bucks or combine them with other technicians to have a pizza party. Each mouse buck was tracked by both the people who gave and the ones who received them. Over 4 mo a total of 367 mouse bucks were given. Of the 73 employees that received those mouse bucks, 60 people also awarded mouse bucks to others. This shows an 82% participation rate. In the laboratory animal industry, where the health and wellbeing of animals are directly proportional to the care given by the technicians, it is essential to implement recognition programs such as mouse bucks.

#### **P122 Making an Academic Animal Research Facility GLP Compliant**

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Good laboratory practice (GLP) animal studies at the Cardiovascular Innovation Institute (CII) are supported by the University of Louisville Office of Research Services. The involvement of the professional and technical veterinary personnel who oversee the CII animal surgical facility and other research resource facilities (RRF) on campus is a critical part of the infrastructure required for conducting nonclinical device testing according to GLPs. Provisions for animal care and animal supply facilities along with general animal care are defined by the Food and Drug Administration (FDA) in 21 Code of Federal Regulations (CFR) Part 58, Sections 43, 45, and 90, respectively. Since AAALAC certification and an effective IACUC are mandatory for academic research facilities, dual compliance with FDA and US Department of Agriculture (USDA) regulations is essential to satisfy both sets of standards. There are many similarities and overlap between GLPs and CFR, Title 9 on Animal Welfare; however, coordinating the efforts of the CII quality assurance (QA) program and that of the RRF operation, takes a total team approach with ongoing dedication and commitment from staff, faculty, and the administration. Veterinary, animal husbandry, and CII QA work together to create, implement, and monitor the necessary policies and procedures to ensure study participants, supplies, equipment (with scheduled maintenance/calibration), and other relevant ancillary support services comply with established standard operating procedures (SOPs). The process also involves ongoing training, extensive documentation and auditing to demonstrate adherence to SOPs established to cover any aspect of animal care from procurement to necropsy including record retention and archiving. The additional level of detail associated with the GLPs is often more labor intensive initially, but translates to enhanced efficiency, improved data integrity, reproducible results, and study reconstructibility.

#### **P123 Incorporation of Flow Meters to Ensure Appropriate CO<sub>2</sub> Euthanasia Techniques**

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Recent research suggests that the most humane method of euthanasia with carbon dioxide (CO<sub>2</sub>) is to fill the chamber with CO<sub>2</sub> at a flow rate replacing 20% of the chamber volume per minute. This method was adopted as a policy by our institution, but the practical application was challenging. We had to translate 20% of the volume per minute into a real flow level for each euthanasia situation. With multiple animal facilities on campus, some with CO<sub>2</sub> tanks, some with CO<sub>2</sub> supply lines, and several with slightly different sized cages, ensuring appropriate and consistent methods required examining each potential situation. We purchased gas flow meters that measured as low as 0.5 L, allowing an accurate low flow setting. Flow meters were attached to both standing CO<sub>2</sub> tanks and counter top mounted CO<sub>2</sub> line spouts. The dimensions of various rodent cages were used to calculate the cages' volumes in liters, and 20% of the cages' volumes. Finally, training and written instructions were provided. Signs that explained the new policy and provided step-by-step instructions for the proper euthanasia method using the appropriate flow rate were posted at each euthanasia station. Hands-on training was provided to research personnel. Staff members who performed CO<sub>2</sub> euthanasia both before and after the modifications report that they see fewer signs of distress, such as rapid movement or circling, jumping, and scratching against the cage walls, during euthanasia. After the CO<sub>2</sub> flow is initiated, animals gradually become unconscious in a smoother manner than before. The modifications our program instituted allow us to verify that a flow rate of 20% volume displacement per minute is used and that we are in compliance with the NIH's PHS policy. Overall, the modifications to the CO<sub>2</sub> euthanasia procedure have decreased the likelihood of pain and have improved animal welfare during euthanasia.

#### **P124 A Committee to Conserve Energy and Resources in Animal Facilities**

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Energy and resource conservation is an issue that needs to be addressed. In general, animal facilities generate a lot of waste, so we decided to look for ways to save energy and resources at our institution. We formed a committee of volunteers from all of the facilities. Our committee is called the Green Team, and we consider ourselves the green eyes of our facilities. Our meetings are held once a month, and our goal is to identify problems and find solutions as well as share some good habits. There are many ways for us to save resources. For example, by cutting off the autoclaves when they are not running, a lot of water is saved. Shortening the cycles on the autoclave and cage washers, always filling cage washers and the racks to the max, and spraying the carts and barrels with a sanitizer instead of running them through the cage washer saves time and energy. In addition, paying attention to the amount of bedding and food that is being used, and, if possible sweeping the floors instead of hosing them down are other ways to conserve energy and resources. Also, in our kitchens/break rooms we use real dishes and silverware rather than disposable ones that would sit in our landfills. There are recycle containers for plastic, glass, and aluminum around the facilities. We ask that everyone recycle plastic grocery bags from home to use in small garbage cans and for small carcasses. The offices have boxes to throw the unwanted paper into, which can also be used as scratch paper. We have a small lamp on our desks instead of having many ceiling lights on, and we turn off computers and other electric burning devices. Cardboard boxes are broken down then thrown in its bin and recycled into bedding. Bubble wrap is given to the labs since there is always a need for it due to shipping or moving. Overall, the Green Team is making progress in spreading awareness about conservation, and our facilities are saving energy, resources, and money. We recommend the creation of a green team of concerned and enthusiastic people at other institutions.

#### **P125 The Uniform Application Program for Postdoctoral Training Programs in Laboratory Animal Medicine**

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As a profession, laboratory animal veterinarians provide expertise in a broad range of areas, including medicine and research as well as program management and administration. In an effort to keep pace with the growing demand for laboratory animal veterinarians with a diverse range of knowledge and skills, both the number and size of postdoctoral training programs have steadily increased. When coupled with the limited number of veterinarians entering the field, training programs are under increasing pressure to attract and quickly secure commitments from applicants. Due to an absence of external pressures, programs have been free to set their own timetable of applicant review and selection, thereby resulting in an uncoordinated and extended selection process nationwide. This has resulted in difficulties for both programs and applicants to reliably identify a good match between the structure and objectives of a program and the interests and career goals of the applicant. The primary objective of the uniform application program (UAP) is to simplify the applicant review and selection process to the benefit of both applicants and training programs. The UAP is specifically tailored to training programs that traditionally select applicants between November and February to begin their training between June and August the following year. In its first cycle (fall 2008 to spring 2009), the UAP established dates for applicant interviews and position offers. It also facilitated continued communications between programs and applicants who did not “match” during the initial process. Judged to be successful by participants, the UAP will continue in 2009 to 2010. Continued improvements will include cooperative advertising by UAP participants, development of a public resource (for example, a website) to disseminate relevant information, creation of a standardized application packet accepted by all UPA participants, and ultimately, establishment of a centralized web-based application processing system.

#### **P126 Development of a Training Program for a Technical Services Team**

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The University of Michigan Unit for Laboratory Animal Medicine Technical Services Team consists of husbandry and veterinary technicians who provide fee-for-service technical support to research laboratories. The team provides such services as dosing (injections, gavage) and blood and tissue collection. When the program was initiated, it relied on staff volunteers that were given the responsibility of seeking technical training as needs arose. As demand for services increased, the need for formal training to increase the number of trained staff, and to ensure proficiency, became apparent. A formal training program was initiated in April of 2008. Issues faced in the development of a training program included, offering and scheduling training at convenient and consistent times, accommodating flexible numbers of attendees, supplying adequate numbers of training animals, standards for proficiency assessment, and documentation of training. Trainees are divided into 3 main groups, beginner, intermediate, and experienced. Beginner technicians must attend an introduction class, which includes basic handling, injection techniques, needle safety, and hands-on experience. Intermediate technicians begin proficiency training by performing various techniques repeatedly until they are efficient and comfortable with individual techniques. Experienced technicians use lab time to practice as needed, particularly when their services have been requested by a researcher. Most lab time is devoted to training rat and mouse techniques, as those constitute the majority of technical requests. Training sessions are offered on a monthly basis, and one-on-one training is provided as needed. Technicians arrive and leave the lab according to their available time and their learning goals. To document training, a log of initial skills and achievements in proficiency is kept on a shared drive, for team managers to use when scheduling services. Since beginning the formal training program, 31 staff members have been trained in a multitude of skills and have been able to fulfill many technical requests with competency.

#### **P127 Sanitation of Rodent Port Charger Stations: Implications for Bacterial Contamination of Drinking Water**

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Water from port chargers necessary for ventilated rack systems is not routinely collected and tested for quality assurance purposes. This study was designed to develop guidelines on how frequently port chargers should be sanitized to prevent bacterial colonization of this equipment. Rodent racks

are flushed daily. Microbiologic testing of water samples is performed quarterly at the source and the level of the line or rack as indicated by room status. Charging stations were tested to determine the cleanliness of port chargers over a 7-d period. Data were collected in a single rodent facility utilizing reverse osmosis, filtered city water. Water samples were collected sterilely from the port chargers in 7 animal rooms containing ventilated rodent racks. Samples were tested before and after lines were changed at the start of each week and then 3 to 4 times a week afterwards for 6 wk. Water was cultured in BHI broth for 48 h and positive samples indicated by flocculent appearance. Positive samples were characterized by gram stain, API 20E, and API 20NE biochemical identification strips. An additional week of testing from 4 rooms was done for bacterial identification. All chargers testing positive were immediately removed along with the connecting hood water lines and sanitized by high pressure chlorine flush, and/or standard cycles in rack washer followed by autoclave. All testing of water at the source and from the racks were negative. From 37 individual room tests, water samples taken at the level of the charger were positive 39% of the time by 48 h and 30% of the time by 7 d. All bacterial species cultured were common environmental, water organisms. Port chargers could offer a potential source of contamination, especially for immunocompromised rodents, and weekly sanitation is recommended for this small equipment.

#### **P128 Continuous Group Housing Standard for General Toxicology Studies as a Form of Environmental Enrichment**

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Scientific research and experience in other facilities has shown that the social housing of laboratory animals in groups improves their wellbeing by reducing stress and promoting the natural expression of species typical behaviors. Keeping that in mind, we recently opened a new laboratory with state-of-the-art primate, canine, and rodent housing to facilitate our enhanced standard of group housing all species used on our general toxicology studies. Our newly designed primate enclosures allow the housing of animals in groups of up to 4, while still retaining the capability to individually house when necessary for certain procedures (for example, overnight urine collection), and behavioral and health reasons. Canines are housed in sizable kennels that accommodate 3 animals per unit with resting boards and the option to allow access to neighboring enclosures. Solid bottom polycarbonate enclosures house rats and mice in groups of up to 5 animals and contain bedding for burrowing and added comfort. All species are group housed continuously (except during the rare occasions when they are separated overnight for individual sample collections). Our initial studies to validate continuous group housing of all species proved to be invaluable approach in problem solving to refine standard operating procedures that involve animal compatibility, data collection, animal identification, animal handling, and cross-contamination concerns. These studies have also generally confirmed some of the benefits to group housing, which include reduced instances of abnormal fecal observations, less aggression towards each other and technicians, fewer animals expressing stereotypical behaviors, and a more rapid acclimation to study procedures. Based on these results, continuous group housing is an effective part of our environmental enrichment program that benefits the social and psychologic welfare of our animals while still retaining sound scientific practices.

#### **P129 Environmental Enrichment Techniques for Laboratory Swine: A Comparative Analysis of Four Cost-Effective Enrichment Methods**

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Environmental enrichment for laboratory animals, including swine (*Sus scrofa*), is recommended by the *Guide for the Care and Use of Laboratory Animals* and should encourage species specific behaviors. In addition, enrichment devices are more likely to be used in the laboratory setting if they are inexpensive, rapid to implement, and easily sanitizable. In order to expand the existing environmental enrichment program for swine at our institution, we evaluated the effectiveness of 4 cost-effective enrichment devices at promoting species specific behaviors. Yorkshire pigs ( $n = 5$  per group) were offered 1 of 4 enrichment devices: a piece of commercial grade rubber hose, a homemade puzzle feeder, blocks of frozen fruit, or blocks of frozen standard swine pelleted diet presented in a water-filled tub. Animals were



observed for 30 min and their behaviors were placed into 5 different categories: snout contact with the enrichment device, oral contact with the device, body contact with the device, nonphysical interaction with the device, or no interaction with the device. These interaction times were compared to pigs receiving the standard enrichment device used at our institution, a large plastic ball. Each enrichment device resulted in increased interaction time compared to pigs receiving the standard enrichment device. These enrichment devices promoted oral and snout contact, which is important for species specific behaviors such as chewing and rooting. Each device cost less than US\$4 to make and could easily be sanitized and reused. Two enrichment devices (rubber hose and frozen standard chow) did not require novel food items and could be used with swine requiring controlled diets for experimental purposes. Overall, this study identified 4 inexpensive and effective enrichment devices that can be easily incorporated into an environmental enrichment program for laboratory swine.

### **P130 Evaluation of Five Bedding Types on Male Nude Mouse Health and Aggression**

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Immunocompromised (nude) mice are an invaluable tool used in many areas of medical research, ranging from physiologic, genetic, and medical studies. One problem that typically presents itself when housing males together is that of increased aggression towards each other. One contributing factor to this may be the bedding material on which they are housed. A study was carried out to measure the effects of 5 different types of bedding on a change in health with regard to aggressive behavior. At day 14 the health scores declined corresponding with sexual maturity (8 wk old). From the results, it is clear that certain beddings produce better health and less aggression in male nude mice. From this study mice housed on thick corncob appeared to show the least amount of aggression. Aspen chip seemed to increase aggression in male nudes.

### **P131 Refinement of Blood Collection Procedures for Polyclonal Antibody Production in Sheep and Goats**

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Repeated blood withdrawals from antibody producing sheep and goats can have an adverse affect on the animals' health over time if specific blood collection guidelines and controls are not employed. A guideline that has been established in our polyclonal antibody manufacturing organization is to collect a maximum of 1% of an animal's body weight in g per bleed. However, animal weights may change significantly over time so that a weight taken prior to blood withdrawal may not be reflective of the animal's weight at the time of bleeding. To guard against this, and thus the possibility of removing more than the 1% of body weight in g per bleed, we developed a manufacturable process in which an animal is immediately identified by radio frequency identification (RFID) as it enters the bleed stanchion and then automatically weighed, while contained within the stanchion. Using a computer program, the system then calculates and displays the allowable blood withdrawal volume, which is based on the animal's current weight. The blood is then immediately collected up to the allowable limit followed by automatic electronic documentation of the animal's radio frequency identification number and dangle tag number, the animal's weight, the blood weight multiplier, and the final blood weight withdrawn. While drawing blood, there is also an audible alarm that alerts the technician when 75% of the allowable blood withdrawal volume has been reached. This refinement in the blood collection process helps ensure the long term health of the production animals and provides for convenient electronic documentation of animal bleeding activities.

### **P132 Developing Enrichment Programs for Human Primates**

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The daily care of laboratory animals is frequently repetitive, tedious, and isolated. Animal care staff is expected to provide a high standard of care, frequently while working alone and wearing uncomfortable attire in en-

closed rooms. Seeing a need to provide opportunities for social interaction and access to a more hospitable environment, our institution has encouraged their animal care staff to participate in a variety of activities to relieve the stress brought about by the working conditions inherent in lab animal care. Inspired by AALAS Tech Week suggestions and activities, our institution has developed an ongoing program of learning and social opportunities, including classes, potlucks, and employee recognition. As a result, employees have been noticeably more content with their jobs and eagerly anticipate the next event. There is a sense of teamwork and renewed enthusiasm that has carried over to the investigators and their staff. We have concluded that an ongoing enrichment program for animal care staff is vitally important to the wellbeing of our animal care department, and intend to continue to seek new ways to stimulate employees' minds and bodies and create an environment that nurtures their development into the best animal care staff they can be.

### **P133 Mouse Norovirus: Dealing with a Dilemma**

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The discovery of murine norovirus (MNV) created a 3-option dilemma for laboratory animal facilities: no testing, reporting, or restrictions; testing, reporting, no restrictions; and no tolerance. Most institutions had no restrictions as MNV seemed to have little effect in mice. This institution tested and reported but had no restrictions. When it was reported that MNV could have clinical significance we changed to a "no tolerance" standard using a 4-phase approach. Detection used the facility's unique sentinel program to determine the extent of the outbreak. During containment, PIs either depopulated or rederived. Positive animals were transferred to 2 rooms so breeding and experimentation could continue until rederivation was completed at an outside institution. Standardized husbandry techniques were strictly enforced. After outbreak, the facility was repopulated with only MNV negative animals. Health status of incoming shipments was carefully prescreened and all animals rederived and from noncommercial vendors were required to be negative after 8-wk quarantine and step-down periods. All facilities were tested to verify negative status. Containment rooms were depopulated, disinfected, and repopulated once rederivation was complete. Negative status was maintained with standardized husbandry techniques and routine health monitoring. Although MNV was detected in all 3 facilities, it affected only 30% of the rooms, PIs, and strains, and was tracked to shipments for individual PIs from specific institutions. One new MNV positive was detected during containment, but was tracked to a break in standard procedures by a single PI. Ten of 12 PIs rederived. After rederivation, 20 of 20 strains tested negative and no positives were detected in the 3 facilities post outbreak. Thus, this institution successfully achieved a "no tolerance" status following an outbreak, but the process took nearly 2 y, cost the institution thousands of dollars, and delayed research. Therefore, dealing with the dilemma of new pathogens by establishing a "no tolerance" standard from the start can prevent outbreaks, saving institutions resources, animals, and lost research time.

### **P134 Visiting Scientist Program: A Path to 3Rs Research**

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Scientists who use animals in research have an ethical obligation to adopt alternatives whenever possible. However, published scientific data about the impact of alternatives on animal research is often not available, making adoption of alternatives difficult. Comparison data is essential to validate alternatives and ease adoption by scientists. Despite interest in alternatives, scientists have little time to pursue these validation studies because of demanding job responsibilities. Dedicated personnel are needed to investigate how alternatives benefit animals and whether they impact the scientific objectives. We are utilizing a visiting scientist program (VSP) to pursue these goals. The VSP is designed to give our scientific staff the opportunity to work in a different scientific area. The goal of this program is to foster an innovative culture through cross-fertilization of ideas, information, and skills, and to provide our scientists with unique development opportunities that can be tailored to their interests. In this case, a scientist with a passion for animal welfare can work with the manager of animal welfare to focus on 3Rs research. The scientist gains new technical skills, exposure to multiple scientific disciplines, and a more balanced perspective about applying scientific principles to animal

welfare issues. This offers new growth for the employee and develops them as a leader for 3Rs initiatives. In addition, there are benefits for the institution; adoption of alternatives enhances animal welfare and can lead to cost savings and efficiencies that streamline drug development. Importantly, research into the 3Rs enables us to highlight our commitment to adopting alternatives to a concerned public. The VSP offers a unique path to 3Rs research that will help to further build our culture of animal welfare.

### **P135 Effects of Alternate Water Sources on Weight Gain, Blood Chemistries, and Food Consumption in Sprague-Dawley Rats and ICR Mice following Ground Transportation**

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Although there are numerous commercial products sold for water source of laboratory animals during transit, limited information is available on their effects on body weight, blood chemistries, and food consumption of rodents during shipment. To evaluate the effects of either a commercially available water source hydration gels or autoclaved potatoes on the health of Hsd:ICR (CD-1) female mice (approximately 21 to 24 g of weight) and Hsd:Sprague-Dawley (SD) male rats (approximately 100 to 124 g of weight) over 5 d of transportation, we measured body weight change, food consumption, and approximate water source consumption during transit and select clinical pathology parameters following transit (Hct, BUN, ALT, ALP, and TP). Additionally, ambient shipping box temperature was monitored using a temperature logger at intervals of every 6 h over 5 d in transit. We found that both rats and mice provided autoclaved potatoes showed minimal dehydration based upon nonfasting blood parameters compared to animals provided ample hydration gels. Weight gain was noted in both mice and rats provided ad libitum hydration gels as a water source. Additionally, rats provided hydration gels as a water source maintained a statistically higher daily feed consumption (approximately 15 g) versus rats provided autoclaved potatoes (approximately 6.8 g). Our results suggest that provision of hydration gels minimizes dehydration and anorexia in rats and supports weight gain in transit.

### **P136 Optimization of an Off-the-Shelf Vacuum Bedding Removal System**

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Off-the-shelf vacuum bedding removal systems automatically draw soiled bedding from a waste funnel through conveyance piping into an exterior waste container. The major benefit of a vacuum bedding system is efficiency of cage wash staff by reducing handling of waste manually. Eliminating manual handling of waste reduces the biosecurity risk to the facility by having to transport multiple plastic bags of waste, gondolas, or other containers through common corridors to a waste container. Vacuum bedding removal systems provide a direct safety benefit by drastically reducing manual lifting of bagged waste; therefore, reducing the risk of a back injury. These systems can also be considered to have a positive impact on the environment, assuming the system eliminates the use of plastic bags which end up adding to the landfill with their extended decomposition time. The downside of utilizing a waste bedding removal system is the fact that it becomes obstructed, from time to time, over the life of the unit. Through experimenting, we were able to optimize the vacuum bedding system by increasing operational run time and automating a manual process of the system. With the goal of maintaining operational run time and eliminating manual processes, maintenance personnel were compelled to identify issues where suggestions could be made to improve the system. System modifications were made to piping, the purge process of particulate collection on the filters and the manual handling of particulate released from the filters. Additionally, removing manual handling avoids the potential biosecurity threat of handling bagged particulate waste. The vacuum bedding system optimization has completely eliminated manual handling, and significantly (approximately by 500%) increased the operational run time of the system. Vacuum bedding system optimization was successfully executed and could be applied to other waste bedding installations.

### **P137 Cage Card Tags Increase the Effectiveness of Rodent Cage Labeling and Improve Communication between Veterinary, Animal Care, and Research Personnel**

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Improving communication between veterinary, animal care, and research staff can be a daunting task when managing a complex university animal care program. It can be difficult to effectively and rapidly communicate information regarding rodent care and husbandry at institutions with multiple animal facilities and large numbers of research personnel. To improve communication in our animal facilities, we have developed a system of small, color-coded, transferable, and reusable cage card tags (approximately 1/8 of the size of a normal cage card) that can be placed directly in front of the animal's original cage card. These tags provide invaluable information about individual rodent cages without obscuring other vital cage card information. Examples of the tags created include: surgery date, special water, special feed, aseptic, date pups found, do not medicate, do not move cage, preexisting health concern, and approved weaning extension. This system has proven to be a simple, cost effective, and extremely valuable method of improving communication and increasing the efficiency of daily animal care duties. The tags have also served as an effective solution to concerns over the use of stickers or taped notes, the residue of which can act as a trap for pathogens and be a source of disease transmission. In summary, the use of transferable, color coded cage card tags has proven to be a simple and inexpensive method to communicate vital information between veterinary, animal care, and research staff.

### **P138 Mouse Models, Common Inbred Strains, and Common Conditions an Animal Technician May Observe**

RD Hyzer\*

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We identify some of the genetic conditions and illnesses that are exhibited by inbred strains of mice. Ten different strains of commonly used inbred mice were chosen to outline the most common and distinguishable conditions that afflict them. Though there may be many health conditions that affect a given inbred mouse, only some of the more health common ones have been identified here. Furthermore, these are the conditions that an animal technician should be trained to observe during their every day work with any of the inbred mice described, for example, hydrocephalus in C57BL/6 mice and light sensitivity and vision loss in the C3H strain. We illustrate 10 of the most widely used strains of inbred mice, that is, the 129, A, AKR, BALB/c, C3H, C57Bl/6, DBA, FVB, NOD, and SJL strain. We aim to help those working with the above mentioned strains to better understand their mice and be able to identify condition that are strain related rather than research induced. By having knowledge about the genetic predispositions of the animals in their care, a technician will be able to offer their animals better care and play a more integral part in the research taking place.

### **P139 Training at a Glance: Use of the On-the-Job Form and Modules to Assist in Training Consistency**

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Within the past year the technical skills required in our department became increasingly complicated and the number of individuals requiring training increased, as did the number of people providing training. All of these factors led to the need for more consistency amongst trainers as well as a formal way for trainees to demonstrate their proficiency. This led us to the development of on-the-job (OTJ) training forms and modules. We designed our OTJ forms, which are living documents, to list the following information: name of the trainee, start and completion date of the training, all necessary prerequisite work, including standard operating procedures (SOPs) and other learning materials, all supportive information relative to the training, the number of animals practiced on, and date of proficiency sign off. A module, outlining what will be covered during the specific training session, accompanies the OTJ form for the trainee to keep for their reference. The module contains the same information as the OTJ form with the addition of a step by step syllabus for the skill being trained. All of these forms are kept on our intranet for easy access by trainers, training mentors, and trainees. The OTJ form and module have provided consistency among our trainers.

For our training team, they also serve as a paper trail to track individual progress and provide accountability, to track animal usage to help assess overall census to support the training program, to ensure all related SOPs and learning materials are known to the trainee and completed prior to finishing the training. Based on the use at our facility, a document such as an OTJ training form and module can be of great help not only to the trainers, but also to the person being trained. Personnel enrolled in training now have a complete overview of what the training session(s) will consist of, along with prework that can be done prior to the training date. As we continue to use these documents, we can explore more data correlation and use them as a tool to enhance the overall efficiency and consistency of our program.

#### **P140 Feasibility Study for the Creation of an In-House Serology Program**

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Surveillance programs for monitoring the health of rodent colonies can be expensive components of an animal care program, especially regarding serologic testing for antibodies. One available option is to develop an in-house serology program in lieu of sending serum to commercial testing laboratories. The primary factor in this decision-making process is whether it will be cost-effective to the institution to create an in-house serology program. In order to determine feasibility, the institution must know the number of sentinel animals per species present in the facility, the frequency of desired testing, and the number of tests to be performed on each of the blood samples. Once the number of tests per year is calculated, the institution can factor in the costs for labor, equipment, and supplies, and then compare annual costs for serologic testing. At our institution, we made the assessment based on an annual sentinel population of 9,500 mice (tested on a quarterly basis) and 500 rats (tested semiannually). The number of individual tests to be performed per year (116,250) was deemed to be manageable by 1 full-time technician whose salary was estimated to be US\$40,000. We then determined costs of supplies (for example, plates, conjugates, control sera) per test. Adding the supplies and the labor, we were able to calculate a 57% reduction in cost by performing tests in an in-house laboratory on a routine basis. Once we determined that routine in-house serology testing was cost-effective, we obtained/purchased the equipment required to operate the laboratory, including a -80 °F freezer, a -20 °F freezer, a refrigerator, centrifuge, plate washers, an ELISA plate reader, an incubator, personal computer, and a pH meter. Over the past several years we have continually examined our costs and found the operation of the in-house serology laboratory to be a cost-effective component of our rodent health surveillance program. In addition to financial benefits, we have the ability to prioritize samples and run tests needed for outbreak control and quarantine release with a 1-d turnaround.

#### **P141 New Performance-Based Testing Procedure for Canopies Installed on Class II Biologic Safety Cabinets**

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This procedure was developed to establish an accurate quantitative test for the canopy exhaust connections used on class II biologic safety cabinets. This study was done in a laboratory with a fixed orifice plate in the exhaust system calibrated to  $\pm 2.5 \text{ ft}^3/\text{min}$ . Helium was injected into the cabinet using an ASHRAE ejector and leak detection was done using a helium leak detector. Cabinet air flow was measured using a direct inflow measuring device (DIM). Three different size class II biologic safety cabinets were tested at 8, 10, and 12 in. sash openings. The exhaust system flow rates were varied from 0% to 150% of the cabinet rated exhaust flow. None of the helium gas injected into the cabinet was detected at the canopy openings when the exhaust rate was maintained at  $7 \text{ ft}^3/\text{min}$  over the cabinets' exhaust flow rating. None of the cabinets lost their A2 flow conditions even though the exhaust air flow varied from 0% to 150% of the cabinets' rated air flow. This new procedure can be used to prove that canopy exhaust connections do not leak gasses into the laboratory. Previous testing procedures used a smoke generator to determine if air was leaking out of the exhaust canopy and, at best, was only a pass/fail test. This procedure will define the air flow rates accurately and is not subject to the personnel interpretation of the direction that the smoke is flowing. In addition, this procedure will show if the cabinet air flow is within the accepted range.

#### **P142 The Suitability of Pine Pellets as a Partial Bedding Substrate for Kennel-Housed Laboratory Swine (*Sus scrofa*)**

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The exclusive use of wood shavings as bedding for kennel housed swine (*Sus scrofa*) tends to be unsatisfactory due to the high expense, failure to fully absorb moisture, and requirement of intensive husbandry measures. Manufactured wood pine product pellets commonly used for equine applications are 0.25 in. in diameter with an average length of 0.75 in. as opposed to the pig feed pellets which are sized at 5/32 in. in diameter with a 0.25 in. length. Thus, animals are easily able to differentiate feed from bedding and seldom ingest any appreciable amounts of the nontoxic pine pellets. In a 30-d test study involving similar kennels of singly housed swine, pine pellets were incorporated into the shavings and then compared to kennels bedded with shavings alone. On a daily basis, waste and soiled material were removed and bedding replenished as needed. Normal rooting activity and hoof pressure from the pigs broke the pellets down into a flaky particulate which combined with shavings and formed a soft loam. This 2- to 3-in. layer proved extremely effective at absorbing moisture and prevented feces from sticking to the kennel floor with an appreciable reduction in ammonia and offensive odors. On average, pigs housed with shavings alone required 2 to 3 times more bedding, storage space, and caretaker involvement than those of kennels containing shavings mixed with pine pellets. An added benefit to the inclusion of pine pellets as a bedding substrate was an increase of additional enrichment for the animals as it promoted the stimulation of natural rooting and nesting behaviors. Thus, when integrated into a routine husbandry program, pine pellets are a suitable partial bedding material for swine requiring less cost and labor than that of wood shavings alone.

#### **P143 Development of a Housing System for *Xenopus* Species with Recirculating and Flow-Through Holding Tanks**

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Various factors were evaluated when planning a conversion from static holding tanks to a multitrack recirculating system designed to house a large *Xenopus laevis* colony. The need to quarantine newly received frogs in the space that had been allocated to the colony posed the single greatest challenge, as recirculating systems reuse tank effluent by filtering (mechanical, carbon, and biological) and disinfecting (UV lamps) system water. Since this process may not consistently remove potential pathogens it was not ideal for newly received frogs. Use of static tanks for quarantine was not desirable either as they require additional space, are husbandry intensive, and water quality fluctuates considerably. The solution was to incorporate flow-through isolation tanks into the recirculating system. Tanks required for isolation would be easily converted from recirculating to flow-through mode by adjusting a pair of valves, which would direct tank effluent to the sanitary system and supply water from an alternative distribution system connected to a timer controlled pump. The alternative supply provides conditioned system water to the shelves containing convertible tanks. Convertible tanks were included on each rack in the multitrack system. Additionally, as 1 of the 3 racks did not require a dedicated sump to collect rack effluent, it permitted the installation of an additional row of tanks that would operate only in the flow-through mode. After calibration, the quantity of water provided to these permanent flow-through tanks is equivalent to the daily 5% water change desirable in recirculating aquatic systems, whereas if all convertible tanks are operated in flow-through mode, a maximum of 13% of system water volume is replaced daily. Total system capacity is 441 frogs, 153 of which can be housed in tanks receiving single pass system water. This flexible system maximizes space utilization and maintains high quality water conditions in tanks operated in both flow-through and recirculating modes while efficiently using water required for replacement.

#### **P144 Keeping Track of an Aging Database**

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As laboratory animals reach maturities desired for certain experiments it becomes increasingly important to keep careful track of the available animals. Our facility has developed a useful system for dealing with those challenges. Every month, cages of mice from various strains are set aside to age. Cages of animals designated for aging purposes are identified with a barcode sticker that contains the principal investigator's name, aging strain number, and a unique cage number. Every aging cage is given a unique number to set it apart from other aging cages and stock cages in that particular strain. All of the information about the aging cage is input into a database application. Within the database, there is a page for every mouse that contains the mouse's strain, strain number, principal investigator, aging cage number, sex, birth date, location in the facility, and current status. If the mouse dies, the page is updated with the date of death and the cause of death. If a mouse is ordered for an experiment, the date of issuance to the investigator is listed as the date of death and "normal issue" is listed as the cause of death. The information from the database is then imported into a web platform for analysis. The aging information can be used to produce survival statistics based on the lifespan of a particular strain. The average age, maximum age, percent mortality, and other data of a particular strain can be determined and cross referenced to other strains with a similar background or all the aging strains in the database. Survival curves for each strain can be laid upon one another to demonstrate the differences in survivability. When all departments involved in handling the aging mice work together, the accuracy and completeness of the voluminous aging data is maximized.

#### **P145 Taking on a Team Approach for Standard Operating Procedures Review**

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Effective standard operating procedures (SOPs) are crucial components of a quality animal care and use program. These documents assure consistency of a facility's training program, provide staff with guidelines for best practices in performing their job, and help assure that regulatory requirements are being met. Effective SOPs must be current in order to accurately describe how to perform a task or use equipment in a real life setting. Periodic review by managers and line technicians is essential to identify and incorporate desirable process improvements. In the past, the departmental quality assurance (QA) manager sent SOPs to management for review and comment approximately 65 d before the due date. This process was inefficient due to slow response by managers and conflicting feedback from reviewers. We implemented an SOP Review Committee composed of a designated staff member from the animal husbandry, technical services, and compliance and management units. The QA manager now sends out expiring SOPs to each of the committee members approximately 70 d before the due date. The committee members gather feedback from members of their units and suggest additional changes based on their own experience; a meeting of the review committee convenes, and each representative brings their compiled comments for discussion. These meetings spark debate, development of new ideas on best practices, and identify inefficiencies and redundancies in the program. We are now able to quickly make consensus decisions and resolve differences by having all responsible parties in the room at one time. As a result of these changes, the turnaround time on remastering SOPs has dropped from an average of 76 d in 2007 to 37 d in 2009. The percentage of overdue SOPs has dropped from 77% in 2007 to 20% in 2009. In the past 2 y since this committee was implemented, we have seen dramatic improvement to our turnaround times. As we continue to move forward and streamline this system, it is becoming easier to meet tight timelines and produce quality documents in the process.

#### **P146 Hose as an Environmental Enrichment Toy for Laboratory Swine (*Sus scrofa*)**

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The use of laboratory swine in biomedical research programs requires that environmental enrichment be provided in order to promote species typical behavior such as chewing. In order to maintain the animals' interest and to

be effective in decreasing destructive behavior, toys should be rotated and used for a limited time each day. We needed to expand our options for environmental enrichment, since several animals destroyed housing pen walls and floors and were losing interest in the enrichment toys currently used. We decided to incorporate into the environmental enrichment program a fire hose at one facility and garden hoses at the other facility. Both types of hoses were cost effective; the fire hose was a donation and the garden hose cost US\$30 per 15.2 m of length. The fire hose was a diameter of approximately 7 cm, and pieces were cut from 0.9 m to 1.8 m in length. The fire hoses were attached to the pen gate or the wall ring in the animals' pens. The garden hoses were 1.9 cm in diameter and cut to 0.5 to 2.0 m in length. The garden hoses were shaped into various configurations and secured with nuts, bolts, and washers to maintain shape and attached to the enclosure gates. Approximately 32 pigs were offered fire hose and 80 pigs were offered garden hose for enrichment. Most animals demonstrated interest in the hoses by chewing on them. Daily monitoring by the animal care staff did not reveal any evidence of injury or ingestion of the hoses. Hoses lasted up to 4 mo before they needed to be replaced. Hoses were easily sanitized within a rack washer or dishwashing machine. This suggests that hoses are a suitable, safe, and cost-effective addition to a swine environmental enrichment program.

#### **P147 Measures against Laboratory Animal Allergy**

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Laboratory animal allergy (LAA) is a serious problem in the occupational health and safety of those working with laboratory animals. In our institute, a case of anaphylaxis occurred 10 y ago, and then a case of severe allergy was found last year. After the latest case, we have strengthened measures against murine allergies, including routine serum antibody tests. In this report, we present an outline of these measures. Our institute has 11 animal facilities, where 45,000 mice, 5,000 rats, and 100 monkeys are housed. A staff of 280 researchers and caretakers have contact with the laboratory animals. The occupational health and safety monitoring of these employees requires periodical submission of a health questionnaire, and serum sampling for cryopreservation before and after working with laboratory animals. The health questionnaire asks about symptoms respondents are concerned about, and allergic symptoms, such as disorders of the eye (itchy, hyperemia), nose (watery, rhinitis), and skin (swelling, rash), as well as asthma and hives. Of approximately 200 of those employees, less than 3% per year have reported allergic symptoms over the last 9 y. A total of approximately 100 occupational health and safety accidents have occurred over the last 11 y in our institute, and 25% of those were related to laboratory animal experiments. We experienced incidences of anaphylaxis due to skin puncture with a syringe used on a rat in 1998, and severe allergic symptoms from a mouse bite in 2008. After the latest occurrence, lectures concerning LAA were given. Furthermore, testing for mouse-specific IgE and rat-specific IgE in the sera has been introduced for employees working with murine animals as of November 2008. Serum IgE was measured using a fluoroenzyme immunoassay. Although only 3% of employees showed allergic symptoms, 13% had murine animal-specific IgE. Thus, a serum IgE antibody test is useful to highlight allergy risk and to prevent LAA.

#### **P148 Filling the Need for Skilled Laboratory Animal Caretakers: Development of a Short-Term Training Program**

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The Dane County Metropolitan Area has experienced high growth in the laboratory science industry. This high growth has led to a need for entry level laboratory animal caretakers, which have become increasingly harder to find. Many technician-level workers seek to advance within the industry, or bored with the nature of this work resign from their positions, which results in costly high turnover rates in the first 6 mo to 2 y of employment. In addition, there are a low number of potential employees with the necessary skills and abilities for entry-level caretaker work in the laboratory field. This lack of skill, partnered with an overall lack of understanding towards using animals in research, makes hiring and retaining animal caretakers difficult. In order to expand the skilled labor pool, local industry

identified key skills that employees need for successful employment. The skills identified include, veterinary medical terminology, animal health and observation, laboratory math, sanitation and communication, laboratory industry overview, and basic computer skills. Laboratory industry professionals partnered with technical college faculty to develop a curriculum that supports the above mentioned skills. This collaboration resulted in an introductory training program that fits on a career ladder leading to high skill/high wage laboratory jobs in the region. This program is a 6-credit, 175-h certificate offered in either 5 or 10 wk cohorts taught by technical college faculty with assistance from laboratory industry professionals. Technical college staff works with local one stops, community-based organizations, kindergarten through grade 12 schools, and developmental education level technical college students for outreach and recruitment into the training program. Thus far over 100 laboratory animal caretakers have been trained, with 60% of graduates securing employment within the first 3 mo of training completion. In conclusion, partnering with industry to develop a short-term training program not only helps industry find qualified employees, but also offers awareness to students about jobs in the laboratory field as well as basic knowledge of research in general.

#### **P149 Evaluation of Housing and Sanitation Practices as Compared to Guide Recommendations Using Laboratory Animal Performance Indices**

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Various considerations exist when determining optimal housing conditions for laboratory animals, including the species and characteristics of animals used and the optimal sized microenvironment for each model. A performance-based approach to establishing cage densities and sanitation frequency allows for appropriate review of practices specific to a given institution. The *Guide for the Care and Use of Laboratory Animals* (the *Guide*) offers recommendations on necessary cage floor space for rodents in relation to body weight, but acknowledges that their spatial needs are complex and not wholly dependent on body weight. The study objective was to assess any significant differences in physiologic parameters measured in C57BL/6NHsd mice and Hsd:Sprague-Dawley (SD) rats housed under conditions that vary slightly from the spacing and sanitation guidelines presented by the *Guide*. Body weight, reproductive indices, behavior, and activity were evaluated along with ammonia levels of the cage microenvironment, observation of cage appearance, use of cage space, and morbidity or mortality of the cohorts. While there was a 5.2% decrease in the growth of male rats housed under experimental conditions, there was a 4.6% increase in the female rat cohorts as compared to *Guide* standards. There was also a slight decrease in the reproductive performance of rats housed according to experimental parameters, but no significant differences among the mouse cohorts. No abnormal behavior was observed among the rats; however, 3 mouse breeders housed under *Guide* standards exhibited overgrooming. No important differences were seen in cage ammonia levels of the rat and mouse cohorts. Mortalities were recorded for a female breeder mouse in the experimental group and a cannibalized mouse litter housed under *Guide* standards. The results of this study suggest that these deviations from the *Guide* husbandry standards had no important effects on the cage conditions, behavior, and reproduction of the rat or mouse cohorts, or on the growth of rats.

#### **P150 Various Applications of Gel Packs for Nonhuman Primates**

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Gel packs have been used in the laboratory industry for over 20 y. Commonly used as water substitutes for rodents, they have more recently been introduced into the nonhuman primate industry; however, their use with primates has not been as popular as with rodents. To determine what might be the cause of this, a series of tests were designed to highlight the benefits and various applications of gel packs for primates: 1) a palatability test was performed to determine how desirable the gel packs were, 2) the gel packs were used in enrichment devices to determine if they would be used for the purpose of enrichment, and 3) the gel packs were provided in transport to determine if they would be used as an alternative to water. Twenty animals (10 *Macaca fascicularis* and 10 *Macaca mulatta*) were selected for the palatability experiment and the enrichment experiment. An additional 10 animals (5 *Macaca fascicularis* and 5 *Macaca mulatta*) were employed for the

transport experiment. Wilcoxon signed-rank test showed that there was a significant increase ( $P < 0.01$  for *Macaca mulatta* and  $P < 0.05$  for *Macaca fascicularis*) in consumption of the gel pack in phase 2 when compared to phase 1 of the palatability experiment. This indicated that primates prefer gel packs after they have overcome their novelty. During the enrichment experiment, it was determined that, *Macaca mulatta* consumed  $66.67 \pm 42.82\%$  SD and *Macaca fascicularis* consumed  $60.00 \pm 31.62\%$  SD of 1 gel pack over an 1 h, which indicates that gel packs are a good means of providing enrichment. During a 5-h transport, *Macaca mulatta* consumed  $80.83 \pm 14.91\%$  SD, and *Macaca fascicularis* consumed  $80.00 \pm 44.72\%$  SD of the gel packs provided as water replacements. This strongly supports the use of gel packs as reliable water source during transport. In addition to these uses, gel packs may serve as an emergency water source, to administer medications, and as an alternative to training treats for animals that are on caloric restrictions.

#### **P151 Preventing Disease Transmission in Rodents through Semirigid Isolator Quarantine**

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Preventing disease transmission in rodents through semirigid isolator quarantine makes importing of rodents from nonapproved vendors available to researchers. With the high demand to use strains of rodents that are not commercially available, the need for a better system of quarantine is on the rise. One of the main components to quality research is providing healthier rodents to the lab staff. Quarantine of rodents from nonapproved vendors helps to eliminate the risk of spreading pathogens to animals that are already established in the facility. In order to prevent disease transmission we quarantine rodents in semirigid isolators for 6 wk before the sentinel rodent is tested for specific pathogens. The sentinel rodent receives bedding from the imported animals for 6 cage changes. While in quarantine we can set up breeding pairs, take tail samples and ear tag, and treat any potential health problems that may arise. As soon as the rodents are confirmed free of the tested pathogens, they are released from quarantine and can be moved to the desired facility and investigators can have access to them. Quarantine of rodents in semirigid isolators is a key component in preventing the introduction of many potential pathogens in our established colonies. Through this process we are providing the investigator a clean rodent model that will not harm all the colonies at the facilities.

#### **P152 A Guide for Starting and Maintaining a Solid Training Program**

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Starting and operating a training program can be a daunting task. Components include orientation of new hires, task-specific training, including hands-on in vivo work, and compliance with IACUC, AAALAC, and federal guidelines. The complexity of the program will vary with the size, organizational structure, and research mission. Efficiency and effectiveness are crucial. We had been maintaining a basic training program that ensured quality animal care along with training for basic to moderate technical skills to support scientific research. Like many other biotech companies, our company's research portfolio expanded to include many new research models and our employee numbers (internal and external) also increased. Therefore, we had to find ways for the training program to keep up with the progress we were seeing. A comprehensive assessment of the needs and goals of the company were critical in order to decide how the program would be enhanced, staffed, and maintained. Our director, associate director, program trainer, and QA department were all involved with the original layout/outline of our training program. By first creating an efficient outline of what our program needed to be, we were then able to fill in the gaps more easily regarding the staffing, documentation, and quality control. During this period of development we were able to establish several tools to ensure that everything had been covered and no important aspects were missed. These tools included an electronic database for record keeping and notification of training requirements, on-the-job training modules that ensured consistency and accuracy, and separate staffing, so that one trainer could focus on the needs of our departmental staff while another could then focus on the scientists' training requirements. We established a checks and balances format to ensure compliance, consistency, accuracy, and, above all, humane care for our animals. Through these processes we have shown

that proper planning, focused teaching, and useful assessment tools allow the process of integrating a new employee into the work environment and ensuring compliance and documentation at an animal research facility to be a smooth and painless process.

### **P153 Optimizing Water Bottle Location for Nonhuman Primate Caging**

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Nonhuman primates (NHPs) on water-restricted protocols require accurate monitoring of fluid intake to ensure proper hydration. Therefore, water bottles must be used instead of an automatic watering system. Manufacturers' location of water bottle holders is important in this regard to make sure those bottles remain in place. Some of our NHP cages are constructed with water bottle holders situated on the sliding center door. This has resulted in problems such as the water bottles being too low, forcing the animals to lie down to drink. Additionally, the sipper tube would get caught on the side of the cage when trying to open the door; this required that the bottle be removed from the cage in order to fully open the door. Not only was this added work, but replacing the water bottles back on the cage could be easily forgotten when returning the animal. Thus, if an investigator left the water bottle off after animal care staff had left for the day, the animal could be without water for up to 17 h. To remedy this, water bottle holders were relocated above the feeders on the side of the cage using simple washers and bolts plus a modification to the T-bar holding the bottle in place. Higher placement of the water bottles meant the animals no longer had to crouch to drink but could sit in a normal position. More importantly, water bottles did not have to be removed from the cages to open the doors. Consequently, the chance of human error was eliminated from the process, and since then no cages have had missing water bottles.

### **P154 Reducing Bedding Dispenser Defects**

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Conducting a process-flow analysis of machine operations can highlight inefficiencies that may lead to substantial productivity improvements via simple solutions. Throughput of mouse cage bottoms was analyzed, beginning at the clean side of a tunnel washer (TW) and through an automated bedding fill station (BFS). Cages moved off the TW conveyor belt bottom side up, slid down a short stainless steel ramp to a mechanical inverter where they were flipped, and dropped onto BFS conveyor rollers to receive a cascading stream of hardwood chip bedding. After installation, the BFS was misaligned with the end of the TW, cage orientation on the TW transport belt was random, and the ramp was bent, occasionally causing unsuccessful flipping. In addition, cages were bouncing excessively off the first few rollers of the BFS as they dropped from the mechanical inverter. For baseline data, any cage arriving at the bedding cascade other than top-side up was recorded as a defect event. It was determined that approximately 10% of the cages (5 of a run of 500) completed the cycle without bedding, requiring their manual retransfer to the BFS entrance. Initial improvements included realigning the BFS to the TW, straightening the ramp, and instructing the TW operator to standardize cage orientation when loading cages onto the transport belt at the dirty side. The resultant defect rate dropped to 2.5% (> 75% improvement). Subsequent enhancements involved common materials purchased from home improvement outlets, such as erecting a plastic curtain over the mechanical inverter (to avoid cage flipping errors) and wrapping the first 4 transport rollers of the BFS with foam pipe insulation (to cushion cages from bouncing). These improvements further reduced the defect rate to 0.06% and saved approximately 690 labor hours of rework annually (equivalent to one-third of a full-time employee's hours).

### **P155 A Simplified Method for the Treatment of Mouse Dermatitis**

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It is well known that C57BL/6 mice are predisposed to dermatitis; however, finding an effective treatment has led to a great deal of frustration. Dermatitis

is painful and disruptive for mice. It can lead to deep infections in the skin, loss of flesh, and more often than not euthanasia. Many different approaches are used to deal with this affliction, but few yield any results. There are treatments that involve the use of topical antibiotics, scrub solutions, powders, or a combination of these. These treatments sometimes end up doing more harm than good, leaving the skin soft, smooth, and open for more bacteria to enter. While doing rounds in the facility with our veterinarian, we were presented with a large group of animals that were suffering with dermatitis. She suggested trimming the nails on the hind feet of the mice; the thought being that if the nails had a blunt tip, as opposed to a pointed tip, they would not damage the skin as severely. The process of mouse nail trimming was begun with a small pair of spencer ligature scissors. The hind feet of mice are usually extended when being scruffed. With the ligature scissors, the nail is grabbed from the underside at the curve. Similar to cat or rabbit nails, the pointed end of the nail is trimmed back, leaving a clean nail that is not sharp behind. Initially, after nail trimming, triple antibiotic was applied to the affected area and it was monitored for improvement. Additional doses of triple antibiotic were applied on subsequent days. The improvement was small. For more severe cases, a course of systemic antibiotics was started. When comparing the outcome of the different treatments, the mice with nail trimming alone showed the most improvement. Most wounds disappeared within 1 to 2 wk, with no reoccurrence. There has been such success with this regimen that it has also been applied to postsurgical mice and rats.

### **P156 A Closer Look inside Rodent Water Valves**

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An increase in the number of leaking water valves was observed in our facility. This resulted not only in flooded cages, but a loss of confidence in the effectiveness of the automatic watering system. In an effort to better understand the cause of the leaks, water valves associated with flooded cages were collected, and the tops unscrewed for inspection. Bedding and rodent fur were found in all of the failed water valves. A study was undertaken to evaluate the effectiveness of the current cleaning procedure, and to help prevent future failure. To begin, new water valves were purchased with the intention of monitoring the amount of bacterial growth and the buildup of debris inside them over time. The new water valves were first washed using an ultrasonic cleaner, followed by immersion in a sipper tube washer, and finally passed through a tunnel washer. They were then placed on 3 rodent racks (A, B, and C). After 1 mo, a sample of 20 dirty water valves was removed from rack A. They were opened and swabbed for microbiologic testing. The water valves were then reassembled and cleaned using the same washing method, after which, they were opened and swabbed again. Lastly, this same group of water valves was autoclaved and tested a third time. These steps were repeated at 3 mo for water valves on rack B, and at 6 mo for water valves on rack C. After 1 mo, debris was visible inside all of the water valves, and over 50% of them tested positive for bacterial growth. Washing decreased the number of water valves with bacterial growth, and autoclaving them eliminated all bacterial growth at any time period. Debris was found in water valves at all time periods, and was detected consistently after washing and autoclaving. In order to effectively eliminate debris, the water valves had to be manually opened and wiped out. The current practice of washing water valves at 6-mo intervals can be maintained, but must always be followed by autoclaving to eliminate bacteria. To determine the potential presence of debris and prevent flooded cages, water valves must be visually inspected during weekly cage changes. In cases when dripping is detected, the water valves must be opened and cleared of debris.

### **P157 Decline in Aggression in Cotton Rats through the Use of Enrichment**

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The cotton rat (*Sigmodon hispidus*) presents a unique susceptibility towards human pathogens and is currently used in studies of human respiratory syncytial virus, adenovirus, and parainfluenza virus. Though valuable, this animal model has earned a challenging reputation with husbandry, veterinary, and research personnel. Cotton rats tend to be hyperactive and aggressive in the lab setting and are likely to leap from their cages or bite



when handled. Frequent fighting between cage mates, particularly males, is often brutal and deadly, compromising study results. Our researchers were concerned about the aggression and fighting in their colony and asked for a solution. An Internet search revealed nothing helpful, so we suggested the addition of cardboard tubes (3.2 in. × 5 in. × 0.30 in.) to the cages for shelter and enrichment. We pair-housed the animals and provided 1 tube per male to prevent competition. The change in the animals' behavior has been overwhelming and our technicians, veterinarians, and researchers are satisfied with the outcome. Prior to the addition of the tubes, it was necessary to handle the rats wearing heavy leather or ISEA level 5 gloves for protection from biting. We now wear latex gloves during cage changing with no more fear of biting than from other common rodents. In 1 y, fight wounds in our cotton rats have decreased by nearly 400% and the animals are typically seen resting peacefully with their cage mates in or near their tubes.

#### **P158 A Novel Method That Increases the Efficiency of Carbon Dioxide Euthanasia in a Rodent Facility**

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Carbon dioxide (CO<sub>2</sub>) is an acceptable euthanasia method for rodents as outlined by the American Veterinary Medical Association Guidelines for Euthanasia. Although combining groups of rodents of the same species into a single chamber is acceptable, NIH guidelines (Guidelines for the Euthanasia of Rodents Using Carbon Dioxide) recommend euthanizing animals in their home cage whenever possible. Using a manifold constructed of plexiglass and flexible plastic tubing, we were able to simultaneously euthanize multiple groups of rodents (rats or mice) in their home cages. The number of cages that can be exposed to CO<sub>2</sub> simultaneously varies depending on the specific needs of the animal care program, and construction of the manifolds can be customized to meet these needs. The manifolds are inexpensive, easy to construct, and easily attached to our existing CO<sub>2</sub> lines. Fill rates were calculated and verified from each line extending from the manifold to ensure the NIH recommended fill rate (a 20% fill rate of 100% CO<sub>2</sub>) to each cage was achieved. Animals were observed in their home cages before and during euthanasia. No change in behavior was observed while being euthanized in their home cage, including dams with litters. Utilizing the CO<sub>2</sub> manifolds we are able to achieve a reduction in time required by personnel to euthanize the rodents. More importantly, we avoid potential stress and social aggression caused by removing rodents from their home cages and combining with unfamiliar groups into a single euthanasia chamber.

#### **P159 Industry and Academia Collaboration Creates Timely and Cost-Effective Preclinical Outsourcing Solutions**

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Global biopharma, chemical, and medical device companies are dependent on contract research organizations for accurate, timely, and cost-effective preclinical outsourcing solutions. They must make significant financial investments to support compliant vivaria, state-of-the-art instrumentation/equipment, and highly trained technical staff. Sophisticated and costly systems are difficult to justify and cost prohibitive for small to midsized companies. Underutilization results in significant inefficiencies and increased costs. Academic institutions leverage several users to justify and support such resources but often require institutional subsidies to operate within the academic environment. Under-used academic resources provide an opportunity for the academic community to increase utilization by working with drug development organizations. However, regulatory compliance, administrative hurdles, timeliness, and intellectual property issues associated with private industry working within an academic environment, often prevent this collaboration from occurring. Progressive US-based research institutions are leveraging in-house capabilities by allowing contract organizations to operate efficiently within their existing business framework. Over the past 11 y, we have developed an academic/private collaboration resulting in increased occupancy of our vivarium, increased staff awareness of government regulatory requirements, and sharing of technical resources. The contract organization has benefited from the availability of the vivarium capacity and access to technical expertise while

operating in an efficient and cost-effective manner as well as utilizing other resources from local academic institutions. This hybrid contract research organization employs dedicated GLP-trained operational staff including study directors, boarded toxicologists, and a management team focused on the timely execution of studies while adhering to strict regulatory compliance. This relationship provides an interface to the drug development industry and leverages the advantages of this emerging business concept with benefits to the academic and private collaborators.

#### **P160 Enrichment: A Technician's Point of View**

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We strive to provide the best care for our animals, and the same should be done for technicians. Technician enrichment can lead to increased self worth, teamwork, productivity, higher job satisfaction, and a lower turnover rate for the facility. Technician enrichment not only deals with providing technicians the tools and education to better themselves, but to also have fun and enjoy their work. One of the ways we recognize technicians is to celebrate. Every time someone passes an ALAT, LAT, LATG certification test or the CMAR series, the Veterinary Medical Unit hosts a celebration in their honor. Birthdays, service anniversaries, holidays, baby/bridal showers are also causes for celebration, and a potluck style get together provides a break from the weekly grind. The group gatherings also provide an opportunity to showcase a team member who has stepped up to help their fellow coworkers. A Technician of the Quarter award is given to the individual who accrued the most team recognition forms. These forms provide a forum for everyone to anonymously thank those who went above and beyond the call of duty. Helping your coworkers not only increases the team atmosphere, but it can also win you a US\$200 bonus. Each year, every member of the staff is presented with the list of recognitions earned. Our facility also encourages technician enrichment with career advancement and participation within AALAS. We provide study groups for certification exams, wet labs, AALAS Learning Library memberships, and sponsor technicians with memberships to both our local and the national branches. The support system provided to our technicians allows them to be productive and enthusiastic members of the team.

#### **P161 Technical Services Offered by the Veterinary Medical Unit**

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Researchers are faced with some great challenges, one of which is trying to answer the question "how can I do more with the resources I have?" The competition for research dollars makes it necessary to use people, time, and money more efficiently. The Veterinary Medical Unit (VMU) at the Portland VA Medical Center is helping investigators do that by offering assistance with various technical services. Instead of having graduate students and postdocs breed and maintain a colony of mice or spend time in a procedure room collecting tissue samples for genotyping, a PI can opt to "rent a tech" from the VMU to complete such tasks. Having someone else complete the routine procedures allows an investigator's lab personnel to do more research. Currently, the VMU has technicians trained in breeding colony management, animal identification (tattoo, ear punch, and ear tag), tissue collection (tail tips and blood collection), body condition scoring, scoring of disease models, surgical assistance, and other needs per request. A Technical Services Team has been established to organize training and refresher courses, delegate procedure requests to VMU staff members, and to identify other procedures or services that could be added to the list of offerings. To take advantage of this service, a PI needs only to fill out a procedure request form and route it through the team's coordinator. The coordinator contacts personnel trained in the requested procedure, and schedules the procedure with the PI and the available technician(s). A technical services fee is billed to the PI in 15-min increments.

#### **P162 The Amazing and Monogamous Prairie Vole (*Microtus ochrogaster*)**

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Constructing and maintaining a breeding scheme for prairie voles has been an exciting and interesting challenge for our technicians. In creating an appropriate scheme, many factors are considered. Husbandry issues, such as temperature, humidity, light cycle, caging type, bedding style, and feed/water administration, are considered. The usual breeding statistics are important: gestation length, average litter size, weaning age, length of breeder viability, and separation age. The unique characteristics of the voles, their aggressive temperament and the fact that they are monogamous breeders, also factor into how to maintain the colony. We use a family scheme based on tracking females, which is designed to maintain an outbred stock of animals. The breeders are held to specific production standards and are replaced if they do not meet or maintain our standards. We relied on previous mating data in setting up the colony boundaries. The vole is a wonderful animal model, and can provide a unique opportunity for technicians eager to handle a new species that is somewhat similar to rats and mice.

### **P163 The Effects of Caging Systems and Cage Locations on Ventilated Rack on Mouse Oocyte Yield and Breeding Production**

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While static and ventilated caging systems are both widely used in transgenic mouse production facilities, the effects of caging on mouse oocyte and progeny production have not been sufficiently analyzed. In this study, we first compared the oocyte yield of FVB and B6 mice on individual ventilated and static isolated cages. Our data indicated that there were no differences. The average oocyte per female in the static versus ventilated caging system was 18 versus 21 in FVB and 31 versus 31 in B6, respectively. We subsequently tested the females' oocyte yield when they were housed in different areas of the ventilated rack (VR). It appears that animals in cages located at the bottom or far right side of the VR produced more oocytes than those animals placed in the middle or top of the rack. To further verify whether the location effect has any influence on mouse progeny yield, FVB and ICR dams, 6 groups per strain at 2 mice per cage, were paired with 1 male of their own strain per cage. The breeder trios were housed at the middle and bottom of VR, and their litters and litter size were tracked for 1 y. Coincidentally, dams housed at the bottom of the VR produced more litters than the dams housed in the middle of the rack. At each cage location temperature (T), relative humidity (RH), light (L), and airflow (AF) were recorded. Temperatures ranged from 75 to 76 °F (average 76 °F), RH was in 55% to 59% (average 56%), light levels were between 6 to 12 footcandles (average 9), and airflow ranges from 29 to 39 CFM (average 34). This information should prove useful for colony management for both transgenic mouse production and general breeder colony maintenance.

### **P164 Paying Tribute to Laboratory Animals in Your Facility**

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During a time in which several large projects were coming to an end, it became apparent that euthanizing animals at their experimental time points was affecting the morale of veterinary and research staff that were not as experienced or familiar with animal euthanasia. More frequently individuals were expressing emotional distress and feelings of guilt over euthanizing the animals they had cared for and worked with over extended periods of time. In response, a plan was established by the animal research facility to develop and present an animal tribute. The purpose of the tribute was to acknowledge the emotional difficulties that euthanasia poses on staff, as well as to remember the lives of all research animals. All research staff members, especially those who work with animals, were invited to attend the tribute presentation, which explained strategies that can be used to decrease euthanasia-related stress and showed similar tributes performed at facilities around the world. Research staff was encouraged to take part in the tribute by sharing their feelings about working with animals and the importance the animals play in their work and medical advancements. A bronze plaque was presented in recognition of the laboratory animals used within the facility and was later mounted in the entryway of the animal research facility. The tribute was well attended by all research staff and

administration. Many attendees sent letters of appreciation and thanked our staff for acknowledging their feelings with such a memorable event. In response to the tribute, many laboratories held their own private meetings to address the feelings of their staff members and to enhance team work. The animal tribute was a successful event, and is anticipated to become a yearly event with increased participation.

### **P165 Identification of Flavor Preference in CD Rats and Hartley Guinea Pigs**

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Decrease in food and water consumption with subsequent dehydration is a common condition in research rodents experiencing physiologic stress such as recovery from surgery and transportation. The identification of palatable flavor additives could be beneficial in the formulation of hydration products for rodents that promote water consumption and subsequently improve hydration. This study was designed to identify flavors and flavor enhancers preferred by guinea pigs and rats. A total of 24 flavors/flavor enhancers were tested in 36 guinea pigs (CrI:HA) and 36 CD rats (CrI:CD(SD)). The flavors were classified as fruits (apple, cherry, grape, orange, raspberry, banana, and mango), vegetables (kale, spinach, carrot, and lettuce), sweeteners (sucralose, HFSC, sugar, honey, molasses, and stevia), oil enhancers (wheat germ, sunflower, peanut, corn, and oat seed), and others (green tea and vanilla). Singly housed animals were provided 2 water bottles, one with a test compound and the other with standard (unflavored) water. Consumption of both flavored and unflavored water was assessed daily for 5 d. Preference of flavored water was assessed by comparing consumption of treated and untreated water for each cage over a 5-d period. The preferred sweeteners were sugar for rats (95.4% consumption) and sucralose for guinea pigs (83.5% consumption). The preferred fruit flavors were mango for rats (70.1% consumption) and orange for guinea pigs (60.6% consumption). Rats preferred spinach (57.9% consumption) as a vegetable flavor, whereas guinea pigs preferred lettuce (64.1% consumption). The preferred oil was sunflower for both species (65.9% consumption for rats and 52.7% consumption for guinea pigs).

### **P166 A Refined Procedure for Short-Term Intravenous Infusion in Canines**

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Many drugs are dosed by short-term intravenous (IV) infusion in order to avoid or reduce adverse events associated with excessively rapid administration. Current industry standards for preclinical IV infusion in canines use surgical models with vascular access ports (VAPs). Implantation and maintenance of VAPs in canines would require resources that could not be justified based on the limited number of infusion studies performed in our laboratory and the relatively short (up to 4 h) duration of infusion required for these experiments. A variety of alternatives to VAPs were examined, including restraint of the canines in slings throughout the infusion or the use of temporary jugular catheters, both of which presented unique challenges. We generated a third alternative, which was simple, noninvasive, and easily reproducible. Male beagle dogs were acclimated to wearing an ambulatory backpack jacket with a lightweight (< 200 g) peristaltic infusion pump housed in the jacket pocket. A temporary transdermal catheter was placed in the cephalic vein and attached to an extension set, which was connected in turn to the pump. The catheter and tubing extension were secured using bandage tape and a stretch net sleeve. The dogs were housed individually in metabolism cages and could be exercised on a leash during the infusion. Using this method, we were able to conduct continuous infusion studies while the dogs were completely mobile. This refinement of standard infusion practice in canines eliminates the need for long term restraint or surgical preparation, thus reducing stress on the canine. More importantly, as the dogs were not surgically altered, they could be used for other studies.

### **P167 A Kininogen-Free Rat Model Useful for Investigating Chronic Inflammatory Diseases**

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The treatment of chronic inflammatory diseases, such as inflammatory bowel disease (IBD) and rheumatoid arthritis (RA), continue to be a challenge to the medical community. Human plasma high molecular weight kininogen (HK) has been recognized as playing a role in chronic inflammation. In order to investigate the role of kininogens in IBD and RA, our laboratory created a unique rat model using spontaneous kininogen-deficient brown Norway rats with a single point mutation that, when backcrossed into a Lewis rat for 6 generations, produced 2 identical strains except for the defective kininogen gene, which codes for poorly secreted kininogen. We studied experimental IBD and RA using kininogen deficient and normal rats and measured markers of angiogenesis, including acute phase proteins. In IBD we followed the clinical manifestation of the disease by gross gut score, histologic inflammation, occurrence of liver granuloma, and white blood cell count. In RA, we followed by body weight, hind ankle diameter, and histologic changes in synovial tissue and cartilage. Results of our clinical and laboratory findings of the proteoglycan-polysaccharide-induced inflammatory bowel disease and arthritis show that all markers were decreased, with a markedly less severe inflammation in kininogen-deficient rats, than in those with normal kininogen. We concluded that the kininogen-free rat model is a reliable, useful model for investigating experimental IBD and RA.

#### **P168 Consistent and Rapid Pathogen Detection Using a Novel qPCR Platform**

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The 3,000 through-hole plate permits the amplification of up to 3,072 individual nucleic acid targets simultaneously in 1 array. This format eliminates the need to multiplex or pool PCR assays prior to the array step. The 3,000 through-hole plate accommodates the use of gene expression assay PCR technology, which has been documented to provide better analytical sensitivity and specificity over conventional gel-based PCR. We investigated the 3,000 through-hole plate to determine its utility for the detection of rodent infectious agents. An array was produced that contained primer and probes representing 20 common viruses and bacteria. Each of the forty-eight 64-hole sub-arrays contained each agent assay in triplicate. Ten-fold dilutions of each target nucleic acid were evaluated in both the 3,000 through-hole plate and a standard 96-well format (duplicate wells). The analytical sensitivity determined for the 3,000 through-hole plate and 96-well format were similar. The 3,000 through-hole plate was resistant to PCR inhibition, associated nucleic acid isolated from feces, and also to high concentrations of test nucleic acid (600 ng/ $\mu$ L final reaction concentration). Potential applications for the 3,000 through-hole plate include virus panels for biologic testing and health monitoring panels for post quarantine, or routine health monitoring of sentinel mice. In addition, we have developed real-time PCR chemistry specifically for the 3,000 through-hole plate that allows researchers to test over 500 samples in 1 d against 56 assays for pathogens and gather high-quality qPCR data. The 3,000 through-hole plate technology provides a simple and rapid method of colony health monitoring.

#### **P169 Evaluation and Comparison of Retroorbital Venous Sinus and Submandibular Venipuncture as Routes for Serial Blood Collection in Mice**

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In serial blood sampling, it is vital to minimize pain, distress, and tissue damage. The submandibular bleeding method in mice was introduced as a procedure to collect blood without anesthesia. Compared to collection via the retroorbital sinus, this method is considered more humane. However, comparisons between the 2 collection methods regarding stress or amount of tissue damage resulting from each have not been published especially for serial blood collection. Serial 25  $\mu$ L blood samples were taken from either the retroorbital venous sinus or the submandibular vein in both anesthetized and unanesthetized CD-1 mice over a 24 h period. Urine corticosterone, packed cell volume, and creatine phosphokinase were measured as indicators of stress, blood loss, and muscle damage, respectively. The statistical

analysis for packed cell volumes concluded that for unanesthetized animals, there was a significant difference in PCV average percentages between the retroorbital and submandibular groups ( $P = 0.0246$ ). Also, there was a difference in PCV percentages between anesthetized and unanesthetized animals in the retroorbital group ( $P = 0.0290$ ). The creatine phosphokinase statistics showed that the unanesthetized animals had higher serum CK levels than the anesthetized animals in the retroorbital group ( $P = 0.0006$ ). The urine corticosterone statistics showed anesthetized animals on average had lower urine corticosterone/creatinine ratios ( $P = 0.0001$ ). Across all animals, there was a difference in urine corticosterone/creatinine ratios between the retroorbital and submandibular groups ( $P = 0.0424$ ). At the conclusion of serial sampling, animals were euthanized and histologic sections were evaluated for morphologic changes. The incidence and severity of microscopic findings from retroorbital collection was greater in unanesthetized mice when compared to anesthetized mice, whereas microscopic findings were slightly more severe in anesthetized mice from the submandibular venous collection method when compared to unanesthetized mice using the same method. In comparison, under histologic review, multiple retroorbital venipunctures appear to result in less tissue damage than multiple submandibular venipunctures.

#### **P170 A Protein Array Platform for Serology Screening of Pathogens in Laboratory Animals**

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Serology has evolved from techniques like hemagglutination inhibition and indirect immunofluorescence assay to more quantifiable technologies like ELISA. We report the development of a new serology method using array as the testing platform. The array system, developed to detect differences in the expression of RNA in samples treated with different agents, is used to study interaction between biologic agents, for example, DNA-RNA, protein-protein, and antigen-antibody. The performance of the array system was analyzed to determine if a multi-sampling, multi-testing technology was amenable to serology testing. This protein array is a modification of the typical microarray technology that permits the detection of antibodies in serum samples. It consists of a solid phase platform that can test 24 samples simultaneously. Each sample is tested for the presence of antibodies against SRV1, SRV5, STLV, SIV, B virus, and measles. At the same time the sample is tested for reactivity to cell tissue culture controls and controls for nonspecific binding. Over 9,000 positive and negative samples were tested to determine specificity and sensitivity of the assay. The qualification of the protein array technology showed a sensitivity of 100% and a specificity of 98.45%. The same samples were also analyzed by ELISA to compare the sensitivity and specificity between the 2 assays. The new protein array platform showed a higher sensitivity than the ELISA, 100% versus 99.26%, and higher specificity, 98.45% versus 95.89%. Also, the variability associated with the slide, by differences in the assay quality within slides and between slides, has an average coefficient of variation of 5.5%, rather than the expected 10%. Advantages to using the array were identified. Testing the sample in the same well against all agents and controls eliminated well related variability. Analyzing the sample against several antigens and controls at the same time allowed for a complete understanding of the nonhuman primate immune state at the time of collection. Samples showed binding only to the specific agent they had an immune reaction against, even in the presence of other antigens and controls. It has higher level of antibody detection than ELISA. Lastly, the array uses less sample volume than ELISA.

#### **P171 Effects of Honey on Feed Consumption and Body Weight of Sprague-Dawley and Obese Rats**

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Honey has many health benefits and has been used as a home remedy for centuries, including in the reduction of obesity. The objective of this study is to find out the underlying truth through scientific experimentation. In a preliminary study, we found that consumption of 10% honey solution reduced the food intake significantly in Japanese quail. Behavior and other activities of those birds including blood glucose level, PCV%, and plasma proteins were normal during that experiment. That study prompted us to investigate on Sprague-Dawley (SD) and obese rats. We fed 2% and 10%



honey (v/v) solution to both SD and obese rats. The SD rats were bred in our laboratory while the obese rats (Zucker, strain code 185, genetically obese rat) were procured from a commercial vendor. Nine healthy male rats were divided into 3 groups (Group I, Group II, and Group III), each with 3 rats (2 SD and 1 obese rat) and were kept in commercially made plastic boxes (L × W × H, 18 in. × 10 in. × 9 in.) individually. The room temperature was maintained at 72 ± 2 °F. Group I (control) received 0% honey in water; Group II received 2% honey (clover honey) dissolved in water (v/v) and Group III received 10% honey solution. All 3 groups were provided routine rat feed ad libitum throughout the 60 d of the experiment. Data were recorded daily on food consumption and body weight. Food consumption in SD rat was maximum in Group I (28 ± 4.38 g/d), followed by Group II (27.4 ± 4.36 g/d), and the minimum in Group III (18 ± 3.21 g/d). In obese rat, it was maximum in Group I (37.2 ± 6.77 g/d), followed by Group II (33.8 ± 9.32 g/d), and the minimum in Group III (24.6 ± 8.02 g/d). The body weight gain were higher in Group I followed by Group II and Group III. In this investigation we found that honey reduces the feed consumption in both SD and obese rats similarly to what we found in Japanese quail. The feed consumption was different ( $P < 0.01$ ) among all groups in both SD and obese rats except for the difference in Group I and II in SD rat. The weight gain was also less in higher honey concentration (10%). This shows that consumption of 10% honey reduces feed consumption and lowers body weight. Further experimentation with higher number of animals is under consideration.

#### **P172 Comparison of Diatrizoate Meglumine and Diatrizoate Sodium Solution to Barium Sulfate as a Gastrointestinal Contrast Agent in Wild-Caught Red-Eared Slider Turtles (*Trachemys scripta elegans*)**

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Red-eared sliders are one of the most frequently encountered chelonians in veterinary medicine and commonly present for gastrointestinal obstructions. Since gastrointestinal transit in turtles tends to be longer than in other animals, a rapid diagnosis of obstruction is difficult. We compared 2 orally administered contrast agents in red-eared sliders with regard to ease of administration, transit times, and image quality. The second objective was to determine if oral administration of a hyperosmolar contrast agent causes significant hematologic changes in these turtles. Fifteen adult red-eared sliders were administered either diatrizoate meglumine and diatrizoate sodium solution ( $n = 7$ ) or 30% w/v barium sulfate ( $n = 8$ ) orally. Dorsoventral radiographs were taken for each turtle at specific time points to assess gastrointestinal transit and emptying times. Prior to administration packed cell volume and total serum protein levels of turtles receiving diatrizoate meglumine and diatrizoate sodium solution were compared to levels at 24, 96, and 168-h after administration and to control turtles not receiving contrast. Barium sulfate had to be administered more slowly (mean = 40s) than diatrizoate meglumine and diatrizoate sodium solution (mean = 20s) in order to avoid regurgitation of contrast. The mean transit and emptying times of diatrizoate meglumine and diatrizoate sodium solution were at least 9 h faster than barium sulfate at all time points except gastric transit. Both agents had a smooth, uniform appearance that outlined the mucosa with well-defined margins within the stomach and proximal small intestine, but dilution of diatrizoate meglumine and diatrizoate sodium solution occurred as it progressed through the intestines. Packed cell volume and total serum protein levels did not significantly differ among the diatrizoate meglumine and diatrizoate sodium solution and control group. When comparing total protein levels among the diatrizoate meglumine and diatrizoate sodium solution group over time, quadratic and cubic trends were statistically significant ( $P = 0.030$  and  $P = 0.028$ , respectively). Administration of diatrizoate meglumine and diatrizoate sodium solution allows for quicker results with only minor hematologic changes in adult red-eared sliders, but visualization of this contrast agent in the lower gastrointestinal tract is likely insufficient for an accurate diagnosis.

#### **P173 The Effects of Salvinorin A on Sedation and Postural Relaxation in Nonhuman Primates**

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Salvinorin A (*Salvia divinorum*) is a plant that seems to produce hallucinations, motor-function impairment, and alterations of consciousness and

perception in humans. Despite wide availability of this hallucinogenic plant, there is little published data on salvinorin A. The present experiment characterized the behavioral effects of salvinorin A in adult rhesus monkeys (*Macaca mulatta*) when injected intravenously. The subjects were 3 male and 3 female rhesus macaques, between the ages of 8 to 14-y-old, weighing approximately 7 to 12.5 kg. Subjects were randomly assigned to 1 of 2 salvinorin A treatment groups (0.01 or 0.032 mg/kg) or the control group (0.16 mL/kg ethanol/polysorbate 80/sterile water). A double blind observational rating study was conducted in home cages. The investigator administered either vehicle or drug in varying amounts, unbeknownst to the technician, who scored the effects of the drugs. A time course design was employed after a single dose administration of material, followed by observations at 5, 15, 30, 60, and 90 min intervals. Two scoring systems were developed to quantify and analyze postural relaxation and sedation. The postural relaxation scale ran from 0, representing no effects, to 4, in which the subject was unable to sit up. The sedation scale ran from 0, representing no effects, to 6. A score of 6 was recorded when the subject did not respond to touch stimuli. Salvinorin A produced forceful dose-dependent relaxation and sedation with fast onset and relatively short duration of action. This finding is consistent with reports of human use. Postural and sedative effects of Salvinorin A were quantified for the first time in macaques.

#### **P174 Ultrasound as a Research Tool**

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The development of animal models of disease based on genomic modification of target genes or the use of transgenesis has become a powerful method to evaluate etiology, progression, and potential response to therapeutic intervention. With the additional complexities that come with these new models, the use of noninvasive diagnostic imaging techniques to follow the disease in vivo is becoming increasingly more critical to their characterization. We have focused on the use of real-time high resolution ultrasound imaging to assess the accuracy and reproducibility in determining the volumes of a number of target organs/diseases over a period of months. Understanding the development of these systems in wild-type animals over a period of time is critical in providing background information onto which disease based determinations can be overlaid. For organ time-course studies, C57/Bl6 or FVB mice ( $n = 5$  per sex per cohort) were imaged on a weekly schedule to determine organ size changes (including kidneys, spleen, prostate, and pancreas) over a period of 3 mo (beginning at 4 wk of age). Even at 4 wk of age, male FVB mice exhibited kidney volumes 18 ± 5% greater than females, with this differential maintained over 2 mo. Percentage kidney growth over the first 2 mo was consistent for both sexes (30 ± 3% from 1 to 2 mo slowing to 10 ± 1% from 2 to 3 mo). Spleen size was more consistent between the sexes over this same time-frame (5% to 8% larger in males than females) and, interestingly, these volumes were maintained from 4 wk to 2 mo (< 3% change). In summary, high-resolution ultrasound is proving to be an important tool in the arsenal available to researchers wishing to follow phenotypic changes in vivo in response to genetic manipulation or disease modeling. We have demonstrated that the technique is accurate, reproducible, and rapid, allowing for detailed analysis of large cohorts over manageable time periods. Sonography is also proving valuable in allowing accurate determination of tumor xenograft volumes (both subcutaneous and orthotopic) where both primary tumors and metastatic lesions can be readily observed.

#### **P175 The Effects of Tank Density on Reproductive Performance in Zebrafish**

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Zebrafish are one of the fastest growing animal models for many reasons, including their embryonic optical clarity, external development, amenability to genetic manipulation, and hardiness. Although great emphasis has been

placed on understanding the genetic control of development in the zebrafish, many questions about optimal husbandry conditions remain unanswered. This research project was organized by the Zebrafish Husbandry Association Working Group on Spawning and Reproduction to help understand the relationship between stocking density and reproductive performance in laboratory-reared zebrafish. This project was designed as a collaboration among 8 laboratories from around the world. Each participating institute dedicated 8 tanks to the experiment and kept fish at all 3 of the following treatment densities with even sex ratios: 3 fish per liter (4 tanks); 6 fish per liter (2 tanks); and 12 fish per liter (2 tanks). After an 18-wk grow-out period all experimental fish were spawned in individual pairwise crosses once every 2 wk for 3 mo. At 1 d post fertilization the number of fertilized and unfertilized embryos was recorded for each successful spawn. Preliminary results show that there is not a significant difference in percent spawning success or clutch size among the 3 treatment groups. The 12 fish per liter treatment group produced the most successful spawns per liter and the most fertilized embryos per liter. Currently the majority of zebrafish laboratories keep fish at stocking densities between 5 and 7 fish per liter. The results of this research project show that keeping fish at stocking densities as high as 12 fish per liter does not have a negative impact on reproductive performance. For some laboratories, increasing stocking densities may be a way to increase efficiency and decrease costs.

#### **P176 Prevalence of *Helicobacter* Species and *Helicobacter bilis* in Mouse and Rat Colonies from South America Animal Facilities**

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Several *Helicobacter* species are known to infect rodents and are associated with gastrointestinal disorders and hepatic diseases. This pathogen is prevalent in mice used for research, inducing changes in experimental results. Previous data from Japan, Europe, and the United States reported a high prevalence of *Helicobacter* infection in mouse and rat colonies. However, there is no information published about the prevalence of these infections in animal facilities from universities of South American countries. We sought to determine the prevalence of *Helicobacter* spp. in barrier-sustained and conventional colonies of mice and rats from South American facilities using the PCR assay. Animals with or without clinical signs from a total of 271 mice, including genetically modified, immunocompetent, and immunodeficient strains, and 51 isogenic and heterogenic rat strains from multiple laboratory animal facilities ( $n = 10$ ) were screened. Bacterial DNA was extracted from fecal pellets using the HotSHOT method. The DNA of *Helicobacter* reference strains *H. hepaticus* (ATCC 51499) and *H. bilis* (ATCC 51630) were extracted using a DNA mini kit. The analysis of *Helicobacter* spp. were performed using a genus-specific primer designed to conserve the region 16S rRNA gene. These primers detect *H. bilis* and *Helicobacter* species (*H. hepaticus*, *H. muridarum*, and *H. rappini*) in a single assay. The genus-specific PCR assay detected *Helicobacter* spp. in 47 mice (17.34%) and *H. bilis* in 66 mice (24.35%) from SPF and conventional colonies. *Helicobacter* spp. were detected in 3 rats (5.88%) and no positive results for *H. bilis* were found in the 51 rats tested. These results have shown the prevalence of a mixed *Helicobacter* infection in 9 mouse colonies. Meanwhile *Helicobacter* spp. were detected in only 1 rat colony. The genus-specific primer was used to evaluate a mouse or rat colony as *Helicobacter* free or infected. Additional tests to identify *Helicobacter* species from positive samples are being performed with species-specific primer.

#### **P177 Microbiologic Monitoring Survey in a Brazilian Laboratory Animal Facility: A Five-Year (2005 to 2009) Report**

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A health monitoring program of laboratory animal for bacterial pathogens is important as some microorganisms species in these animals can interfere with experimental results. This program has been essential to validate the animal quality, the barrier-system efficiency, and the animal welfare as a way to obtain trustworthy and reproducible experimental results. This study shows the frequency of bacterial species isolated from rat and mouse colo-

nies maintained under sanitary barrier-system for a period of 5 y. To search for enterobacteria, small amounts of feces were collected from cecum and grown in culture media MacConkey agar, cetrimide agar, and no inhibition (NI) agar. Swabs from trachea and eye mucous membranes were grown on horse blood agar 5% for detection of *Pasteurella* spp. and other pathogenic bacteria of the upper respiratory tract. All culture media plates were incubated in aerobic conditions at 37 °C for 24 to 48 h, and the isolated colonies were identified in genus or specie levels by biochemical tests. A total of 729 mice and 431 rats of different strains were necropsied and subjected to parasitological examination and serological tests for detection of parasites and serum antibodies for murine viruses and *Mycoplasma pulmonis* infections. Seventeen different bacterial species were identified; the most frequent bacteria found in mice and rats were *Escherichia coli* (87%), *Staphylococcus* spp. (82.2%), *Pseudomonas aeruginosa* (53.3%), and *Proteus mirabilis* (25.5%). The ANOVA test (significance level = 5%) indicated a higher frequency of *S. aureus* (22.7%) and *E. coli* (96.8%) in rats and *Staphylococcus* spp. (89.5%) and *Klebsiella pneumoniae* (5.5%) in mice. Most of the bacteria identified in this study are considered commensal flora found in these species and are not considered pathogenic for immunocompetent animals. However, *K. pneumoniae*, *P. aeruginosa*, and *P. mirabilis* may be opportunistic pathogens for some strains of immunodeficient and genetically modified animals, and their presence can be associated with a husbandry or barrier-system failure.

#### **P178 Development of an Acute Pig Model for Mimicking Hyperdynamic Conditions in Liver Transplant Surgery**

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Patients undergoing liver transplants may develop hemodynamic deviations such as decoupling, in which central (aortic) blood pressure exceeds peripheral (limb) pressures. In order to devise equipment and therapies that accurately monitor and treat these conditions in humans, a porcine model was developed to mimic the clinical picture of a liver transplant, specifically decoupling. The goal was to induce the hemodynamic changes and reperfusion conditions seen in liver transplant surgery without requiring the complex procedures used in traditional hepatic failure models, such as liver removal, chemically induced liver failure, or liver transplant. Previously published liver models involve these more complex procedures and are not ideal because they are lengthy, invasive, and require extensive training. The pig was chosen because its cardiovascular anatomy and physiology is similar to that of humans and its size allows for human-sized monitoring devices and frequent hematologic monitoring. In liver transplant surgery, vascular access to the donor liver is clamped and ligated before liver harvesting. Similarly, in our pig model, liver ischemia was induced by clamping the hepatic artery, portal vein, and abdominal vena cava. The liver was left in place and the clamps removed after a specified time period. Following release of the clamps, toxins normally filtered by the liver had been stored and then released, resulting in post reperfusion syndrome. Post reperfusion syndrome is characterized by hyperkalemia, acidosis, and the presence of vasoactive substances being released from the splanchnic circulation. If not managed properly, this hyperdynamic condition can lead to cardiopulmonary collapse. Methods developed to prevent cardiovascular collapse included slowing liver reperfusion, bleeding toxins from the vena cava rather than letting them recirculate, preventing hyperkalemia through insulin administration, monitoring lactose, pH, and bicarbonate, as well as dextrose supplementation. For acute pig studies, management of potassium and glucose was pivotal in keeping the animal stable, yet maintaining the hyperdynamic state. This model is simple, reproducible, and adequately illustrates the extreme hyperdynamic conditions experienced in liver transplant patients.

#### **P179 Efficacy of a Broad-Spectrum Matrix Metalloproteinase Inhibitor in a Cigarette Smoke-Induced Model of Chronic Obstructive Pulmonary Disease in the C57BL/6J Mouse**

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Chronic obstructive pulmonary disease (COPD), defined as a combination of emphysema and chronic bronchitis, is currently the fifth leading cause of death worldwide and is predicted to be the third by the year 2030. It is primarily caused by cigarette smoke, and there are currently no drugs avail-

able that can halt or reverse the progression of the disease. The inhibition of matrix metalloproteinases (MMPs) is hypothesized to reduce cell influx and prevent destruction of lung tissue associated with COPD. To assess the efficacy of this theory, C57BL/6J mice were exposed to mainstream cigarette smoke for 2 h/d, 5 d/wk for 24 wk, with a broad spectrum MMP inhibitor being administered therapeutically after 12 wk of smoke exposure. Bronchoalveolar lavage (BAL) fluid and lung tissue inflated to a constant pressure were collected at both 12 wk and 24 wk to assess the level of inflammation and alveolar destruction. Morphometric analysis, total leukocyte count, and differential cell analysis were performed to measure the effectiveness of the inhibitor at a dose of 10 mg/kg and 30 mg/kg when administered orally, twice daily. Morphometric analysis revealed that the mean linear intercept, a measure of alveolar destruction, in the inhibitor-treated mice was significantly decreased at both the low dose and the high dose of the compound. The total number of leukocytes, as well as the number of neutrophils, was also significantly reduced at both doses of the drug. Our results confirm that MMPs play an important role in the progression of COPD. The effects of MMP inhibitors on cigarette smoke-induced inflammation and alveolar destruction suggest that MMPs are promising therapeutic targets for COPD.

#### **P180 Improvement of Acute Pain Measuring Method in Neonatal Rat: Subcutaneous Administration of Algesic Substances through Preindwelled Catheter**

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The pain measuring method reported by Kubota et al is an excellent method that can be applied to the quantification of acute pain under the noninflammatory condition. In this method, algesic substances were subcutaneously injected into neonatal rats with infrequent and small spontaneous movements of their body, and then frequencies of their body movements caused by acute pain were measured electrically. However, some problems remained, including contamination of their pain by needlestick, their excitement after being handled for the injection, and difficulties in the measurement during the administration of the substances. While using this method we found some rats with the severe body movements during the injections of saline with no stimulatory agents. To improve the problems, we have successfully developed a procedure where catheters were preindwelled subcutaneously and then the substances were administered remotely through the catheter after the rats calmed down. Using this improved method, which required no handling and no needlestick during the administration, we found rats calm through the experiment and all rats without the pain response during the saline administration. Whereas, the body movements caused by algesic drugs were more frequent in this method than in the conventional method, this improved method has a wide range of measurements. Therefore, we believe that this improved method is especially useful to analyze pain caused by short-acting or weak algesic substances.

#### **P181 Implantable Microfluidic Delivery Platforms for Chronic Administration of Agents for Scientific Discovery and Therapy**

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Diseases are associated with localized manifestations in specific cell types or populations, typically resulting in degeneration and/or tissue reorganization. Investigation and intervention must address the spatial extent of these effects to maximize efficacy with minimal systemic risk. Thus, a new paradigm for studying and treating diseases using microfluidic delivery devices was investigated. Prototype devices were developed for use in mice, rats, and rabbits. Our aim was to demonstrate intraocular delivery in rabbits with the goal of developing a new method for the management of glaucoma. The implantable platform allows delivery of compounds in unaltered format; it consisted of a refillable fluid reservoir, flow regulation valve, and cannula to direct the dose. Refillability of the reservoir with a small gauge noncoring needle will enable chronic use with the added benefit of only requiring a single surgical intervention. The cannula allows direct delivery to targeted sites in the eye, especially difficult to reach regions of the posterior segment, which are the focus of future studies. Surgical shams without the pump and valve were implanted and refilled repeatedly in 4- to 6-wk intervals over a period of 4 to 6 mo with no infection or adverse events. Shams with

the pressure-sensitive valve allowed only forward flow and opened only when a critical pressure was produced by manually depressing the drug reservoir. Valveless drug pumps driven by electrolysis were electronically controlled; both bolus and continuous infusion modes were possible simply by adjusting the current applied. Benchtop studies demonstrated repeatable delivery of specific doses ( $\mu\text{L}$ ) or flow rates ( $\mu\text{L}/\text{min}$ ), even against a pressure head. Drug pumps were implanted acutely in rabbits and 25  $\mu\text{L}$  of phenylephrine was delivered to allow real time observation of pupil dilation (33% vertical and 50% horizontal) to confirm successful delivery. These microfluidic drug delivery systems were fabricated, tested at the benchtop, implanted, and demonstrated in animal studies. Next, wirelessly powered pumps with valves will be fabricated to allow chronic *in vivo* studies using electrolysis driven drug delivery.

#### **P182 Dietary Melatonin and Omega-3 Fatty Acids Inhibit Metabolism in Tissue-Isolated Human Tumor-Bearing Nude Rats *In Vivo* via Receptor-Mediated Signal Transduction**

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Melatonin (MLT) and dietary omega-3 fatty acids (FAs) regulate linoleic acid (LA)-dependent metabolism and proliferation in rodent and human tumors *in vivo*. These agents inhibit tumor LA uptake and conversion to 13-hydroxyoctadecadienoic acid (13-HODE), a lipoxygenase product that enhances epidermal growth factor and insulin-like growth factor I-induced mitogenesis. Here we examine the effects of dietary intake of either MLT (50 mg/d) or 5% fish oil (FO) on the growth and metabolism of established tissue-isolated human PC3 and FaDu cancer xenografts in nude rats. Control PC3 and FaDu tumor-bearing rats ( $n = 18$  per group), maintained on a 5% corn oil (CO) diet revealed tumor latency-to-onset and growth rates, respectively, of 18 and 26 d and  $0.61 \pm 0.03$  and  $0.43 \pm 0.02$  g/d. When tumors reached an estimated weight of 4.2 g, animals were randomized into 3 dietary groups as follows: 5% CO Controls (I), 5% CO + 50 mg/d MLT (II), and 5% FO (III) ( $n = 6$  per group). While control (I) PC3 and FaDu cancer xenografts continued to grow, treatment groups (II and III) of both tumor types began to regress within 2 d of initiation of treatment. Over 2 wk, regression rates for PC3 and FaDu tumor xenografts, respectively, were  $0.14 \pm 0.03$  and  $0.25 \pm 0.05$  (II) and  $0.10 \pm 0.03$  and  $0.18 \pm 0.02$  (III) g/d. Tumor LA uptake and 13-HODE release rates by PC3 and FaDu control tumors (I) were  $1.1 \pm 0.2$  and  $1.1 \pm 0.1$   $\mu\text{g}/\text{min}/\text{g}$  and  $20.7 \pm 2.6$  and  $18.9 \pm 1.6$  nmol/min/g, respectively; however, a complete or nearly complete inhibition was observed in treatment groups II and III ( $P < 0.05$ ). As compared with control tumors (I), tumor cAMP levels, [3H]thymidine incorporation into DNA and activation of MEK, ERK  $\frac{1}{2}$ , and Akt activation were markedly diminished in treatment groups (II and III). These data provide strong evidence that MLT- or FO-induced regression of these LA-dependent human cancer xenografts *in vivo* occur via a cAMP dependent signal transduction pathway, and suggest that these agents may be useful in the treatment and prevention of human cancer.

#### **P183 Two Hypomorphic Alleles of Mouse ASS1 as a New Animal Model of Citrullinemia Type I, an Inherited Urea Cycle Disorder**

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Citrullinemia type I (CTLN1, OMIM# 215700) is an inherited urea cycle disorder caused by deficiency of the argininosuccinate synthetase (ASS) enzyme. Our objective was to describe a new animal model of this rare disease. In this report, we describe 2 spontaneous hypomorphic alleles of the mouse ASS1 gene, barthez (bar) and follicular dystrophy (fold), which serve as an animal model of CTLN1. We have identified bar as an R265C substitution in exon 12 and fold as a T389I substitution in exon 15, both missense mutations corresponding to clinical human ASS1 mutations. These 2 independent mouse mutant alleles interact to produce a range of phenotypes. While some homozygous mutant mice died within the first week after birth, others survived but showed severe retardation in postna-



tal development, alopecia, lethargy, ataxia, and circling behavior. Notable pathologic findings for both mutations were similar to findings in human CTLN1 and included citrullinemia and hyperammonemia, along with delayed cerebellar development, epidermal hyperkeratosis, and follicular dystrophy. Standard treatments for CTLN1 were effective in rescuing the phenotype of bar and fold homozygous mutant mice. We performed linkage mapping and candidate gene sequencing; phenotyping of both mutations, including gross examination; brain and body weights; X-ray; complete necropsy; histopathology; immunohistochemistry; blood chemistry; plasma amino acid; and ASS liver activity. We concluded that bar and fold mutations, as well as their compound heterozygotes, constitute an excellent model for mechanistic and preclinical studies of CTLN1 and other hyperammonemic encephalopathies. Based on our studies, we propose that defective cerebellar granule cell migration secondary to disorganization of Bergmann glial cell fibers cause cerebellar developmental delay in the hyperammonemic brain.

#### **P184 Behavioral Assessment of Laboratory Mice (*Mus musculus*) following Tail Biopsy under Isoflurane Anesthesia**

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Contemporary guidance and anecdotal information suggest that tail biopsy of laboratory mice can be performed prior to 21 d of age without anesthesia, while mice older than 21 d must receive either general or local anesthesia for pain relief prior to biopsy. Our objective in this study was to determine if the administration of isoflurane anesthesia prior to the tail biopsy procedure produced a measurable effect in the behavior of mice ( $n = 196$ ), assessed by lower anxiety, increased exploration, and unchanged activity levels linked to alleviation of post biopsy discomfort. We evaluated 2 strains of mice (C57BL/6 and BALB/c) at 21 to 24 (weaning), 28 to 31 (delayed weaning), and 42 to 45 (adult) d of age. Mice were assessed by acute observation at 10 and 60 min following a 5 mm or sham tail biopsy and then placed on an elevated plus maze (EPM) to assess anxiety responses. Mice were next evaluated for motor deficits in individual locomotor activity boxes for 120 min (30 min light:90 min dark). The tail biopsy procedure significantly increased acute behavioral responses for all ages and strains, regardless of anesthesia, at both acute observation timepoints. Administration of anesthesia resulted in both locomotor and behavioral deficits, decreasing 1) overall activity up to 5 h post procedure, 2) the number of behavioral responses noted at 60 min, and 3) the number of exploratory head dips on the EPM. In addition, mice who received anesthesia spent more time in the closed arms of the EPM and exhibited more stretch-attend behaviors, suggesting increased anxiety-like responses. Similar to previous results from our laboratory, we also demonstrated that animals in the adult age group were most affected by the biopsy procedure, having decreased overall activity following tail biopsy and the most significant increase in acute behavioral responses at 60 min post procedure. The impact of brief inhaled anesthesia on behavior was demonstrable and suggests that anesthetic effects must also be considered when evaluating and interpreting the impact of tail biopsy across various ages and strains of laboratory mice.

#### **P185 *Clostridium difficile* and *Clostridium perfringens* in the Pathogenesis of Idiopathic Enteropathy in Mice**

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Over the past decade, our diagnostic laboratory has seen a rise in the submission of mice with idiopathic enteropathies. Common features of these conditions include sudden death, histologic evidence of enterocyte necrosis, and sloughing with compensatory goblet or Paneth cell hyperplasia and the lack of identifiable known enteric bacterial and viral etiologies. The histologic features of enterocyte necrosis are suggestive of a toxigenic insult to the enteric mucosa similar to that seen in clostridial diseases in a variety of animal species. As a result, we hypothesized that toxigenic *C. difficile* or *C. perfringens* were involved in the pathogenesis of these idiopathic enteropathies. PCR assays to detect the genes of *C. difficile* toxins A, *C. difficile* toxin B, and *C. perfringens* alpha toxin were developed and used to test samples available from each case. Samples tested varied and included paraffin-embedded intestinal tissues, frozen intestinal tissues and frozen feces. No

samples from mice with idiopathic enteropathies ( $n = 11$ ) were positive for either *C. difficile* or *C. perfringens* toxin genes. Based on these results, *C. difficile* and *C. perfringens* do not appear to be involved in the pathogenesis of this syndrome in mice and further studies are needed to determine its etiology.

#### **P186 Muscular Dystrophy Mutations in A and SJL Mouse Substrains**

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Muscular dystrophies of varying severity are caused by mutations in a wide variety of genes. Dysferlin (DYSF) is a plasma membrane protein involved in muscle repair. A disruption in the *Dysf* gene has been shown to lead to a defective muscle membrane repair system causing a number of diseases such as Myoshi myopathy and limb-girdle muscular dystrophy. While dysferlin is most commonly associated with skeletal muscle, recent studies suggest that dysferlin deficiency may play a role in degenerative diseases in cardiac and smooth muscle. Two distinct separate mutations in the *Dysferlin* gene were recently reported in SJL/J and A/J mice: The *Dysf*<sup>frmd</sup> allele carried by A/J mice is a 5 to 6 kb retrotransposon insertion within intron 4 causing a disruption of *Dysf*. The *Dysf*<sup>fm</sup> allele occurring in SJL/J mice is a 141 bp deletion in exon 45. We were interested in the prevalence of these mutations in other mice and performed a PCR study on A/JCrTac mice. *Dysf*<sup>frmd</sup> was detected in A/J mice (supplier 1), but not in A/JCrTac mice (supplier 2). SJL mice and all other strains and stocks tested were also *Dysf*<sup>frmd</sup> - negative. These results indicate that *Dysf*<sup>frmd</sup> must have arisen at supplier 1 after the A/JCrTac substrain became separated from A/J. Mice from the 2 substrains may be an important resource for further investigations in the function of dysferlin because they differ only in this gene. *Dysf*<sup>fm</sup> was found in both SJL/J and SJL/JCrNTac mice, indicating that the mutation occurred before the substrains became separated. B6.SJL-Ptprca/BoyAiTac were also *Dysf*<sup>fm</sup> - positive. All other strains and stocks tested, including Swiss-derived stocks, such as Swiss Webster and ICR, were wild type, indicating that the mutation arose after SJL was separated from those lines.

#### **P187 A Panel of 96 Single Nucleotide Polymorphisms for Genetic Monitoring of Mice**

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Genetic monitoring is necessary to ensure laboratory animals of constant high quality and to avoid mixing up strains that are genetically different but look similar. While microsatellite markers can be used for genetic monitoring, single nucleotide polymorphisms (SNPs) are more easily available in current databases, and are more amenable to high throughput analytic technologies. We have used data obtained from over 2300 SNPs to design a 96-marker panel that can be used for genetic quality control of all of our institution's inbred mouse strains, plus common strains from other suppliers and a variety of 129 mouse strains. In addition to noncoding SNPs, assays for the albino (*Tyr*<sup>r</sup>) and brown (*Tyrrp1*<sup>b</sup>) mutations were included in the panel to provide a check for the coat color, as well as some common spontaneous mutations such as the SCID mutation (*Prkdc*<sup>scid</sup>). Markers were detected on a dual-laser analyzer using a digital holographic code microbeads array and genotyping assay. In short, this is a ligation-extension type multiplexed assay with post ligation PCR amplification and fluorescent readout. The panel was shown to reliably differentiate all major mouse strains and substrains at our institution. For example, 129P3 and 129S6 strains are differentiated by 10 markers, C57BL/6 substrains by at least 3, A/JCrTac and BALB/c by at least 10, and 129S6/SvEvTac and C57BL/6NTac by 49 markers. The 96-SNP genetic monitoring panel will allow for genetic quality control of laboratory mice at a higher level than previously possible.

#### **P188 Establishment of a Novel Method of Focal Cerebral Ischemia Rat Models**

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Focal cerebral ischemia rat model plays an important role in stroke research, but high mortality and unstable infarction volumes remain problems. We compared 3 surgical methods and evaluated the mortality rate, neurologic

evaluation score, infarction volume, and length of procedure. We randomly assigned 60 rats into 3 groups, each performing a different surgical method. Group 1: the common carotid artery (CCA) and external carotid artery (ECA) were ligated during the procedure and the internal carotid artery (ICA) was clipped temporarily by artery clamp; when plastic suture was inserted from CCA, artery clamp was removed. Group 2: CCA and ECA were ligated; superior thyroid artery and pterygopalatine artery were exposed but not ligated; silk suture was used to elevate the ICA; plastic suture was inserted into ICA and crossed over the initial point of the pterygopalatine artery with the help of a microscope. Group 3: CCA and ECA were ligated and ICA was elevated by silk suture; pterygopalatine artery was not exposed, but suture's proximal part was pressed to make the suture's round distal end elevated when plastic suture was inserted, and then pass the initial point of the pterygopalatine artery. The distance of plastic suture inserted for all 3 groups was the same, 1.8 cm. Mortality, neurologic score, infarction volume of ischemia, and length of procedure for all 3 groups were compared. After 48 h of surgery, the mortality rate of groups 1, 2, and 3 was 40%, 20%, and 10%, respectively; the difference between group 1 and group 3 was significant ( $P < 0.05$ ). One week after the procedure, 2 rats were dead in group 3, and 5 and 4 were dead in groups 1 and 2, respectively. The mortality rate of group 3 was significantly lower than that of groups 1 and 2 ( $P < 0.05$ ). The difference of NSS evaluation score among all 3 groups was not significant, but the score of each rat in group 3 was more stable than rats in the other 2 groups. The average infarction volume 24 h after the procedure was 431.6 mm<sup>3</sup>, 413.3 mm<sup>3</sup>, and 401.7 mm<sup>3</sup> for groups 1, 2, and 3, respectively; there were no significant differences among the 3 groups. The length of procedure was significantly shorter for the third method (17.5 min for group 3 versus 50 and 40 min for groups 1 and 2, respectively;  $P < 0.05$ ). Using the third method can shorten the time and improve the success of surgery and produce a more consistent and repetitive focal cerebral ischemia model.

#### **P189 Isoflurane Does Not Alter the Brain Distribution of Reference Compounds and New Chemical Entities in the Rat**

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Penetration of drugs through the blood-brain barrier is an important criterion in CNS drug discovery. Drug concentration in the brain and the brain/plasma ratio are key parameters used to assess the extent of CNS penetration. To obtain rat brain tissue quickly at an accurate time point following dosing, decapitation by guillotine is typically used. In line with our Institution's commitment to the 3Rs, a refinement of this procedure was warranted, as the technique may be perceived as unpleasant to carry out in conscious animals and can, therefore, limit the staff available to perform these experiments. The objective of this study was to evaluate if preterminal isoflurane anesthesia could alter the CNS penetration of known reference and project compounds. Nine reference compounds and 8 new chemical entities (NCEs) representing several chemical series and brain/plasma concentration ratios, reflecting low ( $\leq 0.2$ ), medium ( $> 0.2$  and  $< 0.8$ ), or high ( $\geq 0.8$ ) CNS penetration, were tested in naïve, male CrI:CD rats with and without preterminal anesthesia (5% isoflurane for 2 to 5 min) at 0.5 to 2 h post IV or PO administration. Trunk blood was collected to generate plasma, and the whole brain (without pons) harvested. Plasma and brain homogenate were stored frozen until analyzed by LC-MS/MS, and brain/plasma concentration ratios were calculated. For the NCEs, no differences were observed between the anesthetized and unanesthetized animals in brain ( $P > 0.916$ ) or plasma ( $P > 0.958$ ) concentrations, or brain/plasma ratios ( $P > 0.144$ ). The same was observed for the reference compounds. Our results demonstrate that isoflurane does not confound the estimation of brain distribution under these experimental conditions. Furthermore, the results may be of interest when refining methodology of CNS-penetration experiments for continuous improvement of animal welfare.

#### **P190 Treatment Efficacy, Antibiotic Susceptibility, and Provocation Testing of *Corynebacterium bovis*-Infected Athymic Nude Mice**

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*Corynebacterium bovis*-infected athymic nude mice primarily present with transient hyperkeratotic dermatitis. Our research was motivated by disease outbreaks characterized by increased mortality. We evaluated various treatments to reduce the duration of clinical disease or to eradicate subclinical infection. We also evaluated susceptibility of *C. bovis* to antibiotics pre and post amoxicillin treatment and conducted provocation testing in an attempt to replicate severe disease. Four treatments, including amoxicillin diet (1200 ppm; 200 mg/kg), pen-strep topical spray (2500 units/mL penicillin; 2500 µg/mL streptomycin) in sterile water, sterile water topical spray, and no treatment, were evaluated. In unmanipulated mice ( $n = 60$ ), no significant difference in disease duration was detected between groups. Eradication of *C. bovis* from subclinically infected athymic nude mice was explored with either 4 ( $n = 15$ ) or 8 wk ( $n = 22$ ) of amoxicillin diet treatment (1200 ppm; 200 mg/kg). While 2 mice that received 4 wk and 3 mice that received 8 wk of treatment remained *C. bovis*-free for at least 252 and 174 d after treatment, respectively, amoxicillin treatment was found to be ineffective in clearing infection from most mice. Antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method was conducted on isolates from cages ( $n = 16$ ) containing *C. bovis*-infected mice pre and post amoxicillin diet administration. Subculturing of the isolates elicited both a small and large colony type with different antibiotic susceptibilities. The large colony type was susceptible to most antibiotics while the small colony type variably demonstrated reduced susceptibility to several antibiotics, including ampicillin and trimethoprim sulfa. Lastly, provocation testing was conducted in athymic nude mice ( $n = 14$ ) with 50 mg/kg cyclophosphamide IP every 48 to 72 h for 90 d. Immunosuppression resulted in prolonged clinical disease that waxed and waned without mortality. These findings suggest that treatment of clinical disease in unmanipulated mice is unrewarding, eradication of bacterial infection is difficult, and that severe disease associated with *C. bovis* is likely multifactorial.

#### **P191 Mice Immunization against Larval Ascariasis**

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Animal immunization against parasitic infections, especially *Ascaris suum* infection in pigs, is the focus of numerous studies. It is known that helminthes are among the most potent stimulators of immune responses. According to some researchers, pigs do not demonstrate specific resistance towards migrating ascarids when passively immunized with blood serum of infected pigs, while inoculation with infective stages of *A. suum* or its antigens in animals induces resistance against oral challenge with infective *A. suum* eggs. Besides, immunoglobulins are present in serum of pigs immunized against *A. suum*. We investigated potential immunogenic activity of raw extract of adult *A. suum* in white mice as a model for pig ascariasis. At the first stage, a complex antigen was separated from adult *A. suum*. Tests of the prepared complex of ascarids antigen, show that high dose injection results in anaphylaxis in mice with persistent liver and lungs edema and local necrosis in the injection place. Blood pattern is characterized by significant leucocytosis (19,000 to 24,000 mm<sup>-3</sup>), nuclear left shift, eosinophilia, and monocytosis. According to preliminary data, migration and further development of *A. suum* larvae in mice organism are inhibited in immunized mice. In low dosage, injection does not result in anaphylaxis, and serious health disturbances in mice are not noticed. Larval migration and further development in mice organism are also inhibited in mice immunized by lower doses of antigen as well as after infestation with higher doses of *A. suum* eggs when immunized by middle doses of antigen. Besides, the larval development is also inhibited in mice after infestation with *Toxocara canis* eggs when immunized by middle doses of swine ascarides antigen. Thus, there is probably a cross immunity against different species of ascarides. Single subcutaneous injection of complex *A. suum* antigen results in immunologic changes in mice organisms with production of specific antibodies, which could be detected by means of agglutination reaction. We conclude that the developed preparations for specific prevention of the disease appears to be rather efficacious and highly immunogenic.

### **P192 Establishment of Experimental Avascular Necrosis of the Femoral Head in Nude Rats**

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Avascular necrosis of the femoral head (AVN) remains a major unsolved problem in orthopaedic surgery of the hip. There is no reliable animal model of AVN for the evaluation of new therapeutic approaches such as human stem cells. In this study, we tried to establish an animal model of AVN using immunodeficient animals by surgical methods. Nine 6-mo-old male athymic nude rats were operated by vascular deprivation method under general anesthesia using zoletil/xylazine. Through a medial approach, the joint capsule was transected. The ligamentum teres was cut and the femoral head dislocated. The periosteum of the femoral neck was stripped using a number 11 blade and was burned with bipolar electrocautery. After reducing the femoral head, the joint capsule and muscles were sutured. The femoral heads of the nonoperated left hip served as control. For evaluation methods, we performed the X-ray, micro CT, MRI, gross observation, and histologic assessment at 2, 6, and 10 wk after operation. No radiologic signs of osteonecrosis were seen at any weeks postoperatively. Micro CT images could not evaluate the morphologic changes between operated femoral head and control. Compared to control, signal intensity decreased mildly in operated femoral head on contrast-enhanced MR images. And operated right femoral heads looked paler than control at necropsy. In H&E stain of the femoral heads, there were empty lacunae at epiphysis and pale bone marrow with decreased cellularity at 2 wk after operation. At 6 and 10 wk, we could observe empty lacunae and partial new bone formation at epiphysis with pale and fibrous bone marrow. In this study, we established the surgery-induced AVN model in nude rats and verified it by MRI and histology. Our animal model using immunodeficient rats might be useful in preclinical studies evaluating the effects of human cell therapy for treatment of AVN.

### **P193 Baboons: Assisted Reproductive Technologies**

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Old World primates like macaques (rhesus, cynomolgous) are closely related to humans, and development of assisted reproductive technologies (ART) have been crucial to verify the complex process of fertilization in humans largely performed on leftover and subprime clinical specimens after infertility management. To extend reported macaque observations and provide the underpinnings for expanding nonhuman primate research resources for reproductive, developmental biology and regenerative medicine, we report here on protocols for ART in baboons, including 1) oocyte collection after exogenous hormonal stimulation, 2) intracytoplasmic sperm injection (ICSI) for fertilization, 3) preimplantation development to blastocyst stage in various culture media, and 4) embryo transfer (ET) techniques for pregnancy establishment. We show that follicular recruitment with recombinant human hormones produced high oocyte yields but only about 1 of 3 mature for fertilization. High fertilization rates (>80%) are routinely observed after ICSI. Development to the expanded blastocyst stage is lower than reported in macaques, regardless of tested culture media, suggesting optimal culture conditions await further analysis. Baboon ETs performed by either midventral, laparoscopic transfer, or intrauterine transfers were performed, with numerous early pregnancies by sex skin coloration observed. However, only a single aborted implantation event was documented, with pathology reports suggesting the presence of fetal and placental tissues. Animal stress factors (that is, change in habitation, animal manipulations, and familiar cohorts) may contribute to premature loss of pregnancy establishment. Collectively, although protocols for baboon ART are not yet fully optimized, having another nonhuman primate to bridge the intellectual and biomedical gaps between rodents and humans will enrich the available research resources for reproductive, developmental, genetic, and regenerative medical studies.

### **P194 Rat Telemetry for Cardiovascular Safety Assessment: Strain Influence on Drug-Induced Hypertension**

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The discovery of new drugs with undetected cardiovascular side effects could have hazardous consequences. The effect of new chemical entities, like the sphingosine-1-phosphate receptor agonist FTY720, on mean arterial pressure (MAP) and heart rate (HR) can be explored in conscious rats by using telemetry devices. Identifying rat strain differences may be a critical factor in translating data between Lewis rats used in screening assays with Sprague-Dawley (SD) used in toxicology studies. Six female Lewis and 6 SD rats were implanted with abdominal aortic radiotelemetry implant. MAP and HR from free-moving, single housed, conscious rats were recorded continuously for 26 h. Baseline were determined during a 2 h pretreatment period and compared to MAP and HR values obtained after dosing. Both rat strains were dose orally either with vehicle or FTY720 at 1, 3, and 10 mg/kg (mpk).  $\Delta$ MAP increased dose-dependently from baseline values of  $110 \pm 10$  to  $109 \pm 1$ ,  $110 \pm 1$ ,  $116 \pm 2$ , and  $133 \pm 1$  mm Hg, in Lewis rats receiving vehicle or FTY720 at 1, 3, and 10 mg/kg, respectively.  $\Delta$ HR decreased dose-dependently from baseline values of  $329 \pm 12$  to  $321 \pm 12$ ,  $305 \pm 3$ ,  $289 \pm 5$ , and  $268 \pm 6$  bpm, in Lewis rats receiving vehicle or FTY720 at 1, 3, and 10 mg/kg, respectively. FTY720 induced the same extent increase in MAP and decrease in HR in SD rats. Effects occurred 2 to 4 h in accordance with maximum capacity (Cmax). In conclusion, FTY720 induced a dose-dependent increase in MAP in conscious rats from either strain. The HR reductions appear to be reflex-driven, since they occur at doses that also increase MAP.

### **P195 Monitoring Audiogenic Seizures in *Peromyscus***

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Mice of the genus *Peromyscus* are among the most abundant mammals in North America. They are not closely related to either *Rattus* (rat) or *Mus* (mouse) and cannot hybridize with either of these species. Documented in 1935 by LR Dice, seizure-susceptible deer mice when subjected to auditory stimuli, may whirl rapidly, become stuporous, or exhibit other abnormal behaviors. Early testing methods to identify audiogenic seizure sensitive animals included using the sound of jingling keys or bells. We have developed an exact, reproducible testing protocol using a recorded white noise and HI-FI amplifier and speakers. Eight animals were subjected to white noise for 2 min starting at 60 dB. After 5 min of rest, the intensity was raised by 5 dB. We have determined that seizures in *Peromyscus* are induced at 105 to 108 dB, which is significantly lower than those reported for audiogenic seizure sensitive mice (125 to 140 dB) and rats (135 dB). Sensitivity to seizures as measured by the time elapsed between the onset of the sound and clonus varied among animals (15 to 80 s) consistent with the fact that these animals are random bred. The developed system can be used to study the effect of proven or experimental anticonvulsants.

### **P196 Bronchoscopic Segmental Delivery and Bronchoalveolar Lavage in a Canine Model of Lipopolysaccharide-Induced Pulmonary Inflammation**

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Approximately 40 million people suffer from asthma and COPD. In the development and early screening of new drugs, proof of mechanism models are gradually replacing disease mimicking models. Similar proof of mechanism models are also used for screening studies in humans. We developed a canine pre-clinical proof of mechanism model using segmental delivery of lipopolysaccharide (LPS) to induce local neutrophil infiltration without undesirable systemic effects. Dogs ( $n=24$ ) were placed under general anesthesia and a flexible bronchoscope was passed into the trachea. The cranioventral most lung segment was identified and a gastric wash pipette advanced into the segment where 2ml of LPS (4 ng/kg) was instilled. The contralateral lung segment was used as control in the same dog, with saline instilled in the same manner. Bronchoalveolar lavage (BAL) was performed 4–6 hours



later. Each lung segment was lavaged with 2 × 20 ml saline washes and the lavage fluid was analyzed. Dogs were monitored during and immediately following the procedure for body temperature, respiratory rate, SpO<sub>2</sub>, ECG and non-invasive blood pressure. Further monitoring of heart rate, respiratory rate and effort, body temperature, and food consumption was also performed for 3 days post procedure. Lavage fluid cell counts showed greatly increased neutrophil counts in the LPS stimulated lung segment with no significant changes in the control segment (15.2 ± 5 × 10<sup>6</sup> versus 1.9 ± 0.6 × 10<sup>6</sup>, respectively, *P* < 0.05). There were no significant changes in any of the monitored parameters during the procedure or in the following days. This technique provides a safe, reproducible model of pulmonary inflammation for use in early drug candidate selection. Local delivery of LPS allowed for local induction of neutrophilia with no measured systemic effects of the toxin at any time, and by using the contralateral lung segment as a control, the number of test animals is greatly reduced.

#### **P197 In Vivo Kinetics of Thermally Cross-Linked Superparamagnetic Iron Oxide Nanoparticles in Rats**

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Many different types of nanoparticles have found extensive use in the cosmetics industry and in medicine. In biomedicine they are used to improve contrast in ultrasound and MR images. However, some of them still have toxicity potential in the body, without degradable properties. After delivery, it is desirable for the nanoparticles to be removed or metabolized, ideally without any toxic side effects, from the body. In this study, to gather the information on absorption, distribution, metabolism, and excretion of the nanoparticles, in vivo kinetics of thermally cross-linked superparamagnetic iron oxide nanoparticles (TCL-SPION) was investigated. An emulsion of TCL-SPION (15 mg/kg BW in saline) was pulse injected in a caudal vein of the male Sprague-Dawley rats. The time of the dose is designated 0 h of day 0. Animals were housed in metabolic cages. Time course serum and urine were examined for iron oxide using the guanidine-ferrozine method and ICP-AES method throughout day 0 to 28 with different intervals. The iron deposition in the tissues was examined by combination of Prussian blue and Perls stain method. Serum iron levels decreased in a time dependent manner. The level of serum iron at day 28 was as low as approximately 10% of 0 h. Urine iron levels increased in a time dependent manner. The level on day 28 was 2-fold higher than 0 h. Iron deposition was observed in the parenchymal cells of liver 1 h after injection and lasted until day 28. In spleen, B cell area was stained blue within 30 min and also lasted until day 28. In lung tissue, positive stain was shown in bronchiole areas from 1 to 2 h after treatment. However, no positive stain was detected within 8 h of injection. In kidney, iron deposition was detected in glomerular arterioles from 2 to 12 h of injection. Long time stay of TCL-SPION in certain tissues after intravenous injection suggests that once the target cell has been identified, TCL-SPION might be useful as a carrier of therapeutics for the treatment of diseases.

#### **P198 Natriuretic Peptide Levels for Early Endpoint Determination in a Canine Model of Induced Cardiac Failure**

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The canine tachypacing model is a challenging, complex system for studying human congestive heart failure. Establishing a balance between sound scientific results with minimal clinical sequelae can prove difficult when attempting to establish objective study endpoints. One method of refinement of this model is measurement of biomarkers, such as plasma natriuretic peptide levels, to follow progression of cardiac disease and clinical signs. ANP (atrial) and BNP (B-type/brain) natriuretic peptides are useful biomarkers in diagnosing heart failure, monitoring progress of ventricular dysfunction,

and assessing efficacy of therapeutic interventions. To determine if natriuretic peptide levels correlate with severity of clinical parameters in the canine tachypacing model, we evaluated natriuretic peptide levels together with clinical examination, echocardiography, and other measures of cardiovascular function. Ten dogs were instrumented with pacemaker implants and chronically tachypaced for 6 wk. Baseline and weekly post instrumentation measurements included CBC, chemistry, plasma ANP/BNP analysis, physical examination, echocardiography, ECG measurement, blood pressure determination, pulse oximetry, and cardi thoracic radiography. All dogs showed increases in both ANP and BNP levels throughout the study, although BNP had less variability and was a more sensitive indicator of end stage congestive heart failure. The increase in BNP over time corresponds with a decrease in cardiac ejection fraction, increase in left ventricular end diastolic and systolic volumes, increase in abdominal girth, and worsening of pulse integrity and respiratory parameters. These findings demonstrate that elevations in natriuretic peptide levels occur alongside changes in select clinical and echocardiographic parameters. Natriuretic peptides are also useful as noninvasive methods for determining heart failure progression because they are both diagnostic and predictive of impending cardiac sequelae. A mathematical model is currently being developed to incorporate BNP and other cardiovascular data into a practical formula for early endpoint prediction in this canine heart failure model.

#### **P199 Comparison of Rectal, Transponder, and Telemetry Thermometry for Collection of Body Temperature in Dogs, Monkeys, and Rats**

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Measurement of body temperature is an important part of veterinary medicine and a routine parameter collected on toxicology studies. With increased use of transponders for identification and transponders capable of recording body temperatures, a less invasive and more efficient method may be available. Transponders were implanted in dogs (5 per sex), monkeys (5 per sex), and rats (10 per sex) previously implanted with telemetry devices. An additional transponder was implanted in a separate location to evaluate the potential for temperature changes that could be related to transponder implantation location or migration of chips after implantation. Nearly simultaneous temperature measurements were recorded from the transponder (both locations), rectal, and telemetry thermometry devices for each animal. Data were collected for a 10-d period in the morning (0700 to 0800), mid afternoon (1300 to 1400), and at night (2000 to 2100) to evaluate the ability of each collection method to detect expected circadian changes in body temperatures. In addition, animals were administered compounds to decrease (xylazine) and increase (*E. coli* lipopolysaccharide (LPS)) body temperatures. Following administration of xylazine all methods were able to detect lowering of body temperatures at 4 h post dose for the monkey (3.0 to 4.6 °C) and rat (1.3 to 2.3 °C), and 30 min post dose for the dog (0.4 to 0.9 °C). Following administration of LPS all methods were able to detect a decrease (0.4 to 1.4 °C) followed by an increase (0.5 to 1.6 °C) in body temperature at 4 h post dose for the dog, a biphasic temperature increase 30 min (0.4 to 0.8 °C) and 24 h (0.9 to 1.2 °C) post dose for the rat, but no response was detected for the monkey. Temperature variations related to transponder location were observed in all species with greatest variations in the monkey and dog. Although measurements varied by method, the transponder detected similar patterns of normal as well as chemically induced changes with the rectal and telemetry methods. Based on the results of this study, the transponder is an acceptable means of collecting body temperatures in the rat, dog, and monkey.

#### **P200 Comparison of Diagnostic Testing Methods for *Giardia duodenalis* in Domestic Sheep (*Ovis aries*)**

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Sheep are commonly used in biomedical research for modeling of human cardiovascular disease. There is potential for transmission of zoonoses from sheep during routine husbandry and handling. One zoonotic pathogen of concern is *Giardia duodenalis*, commonly diagnosed worldwide in humans and livestock. Prevalence of giardiasis in sheep has been reported to be as high as 68%. The standard for diagnosis is visual examination of cysts or

trophozoites in feces by microscopy; however, limitations include time, labor, and potential false negative results due to intermittent shedding of the organism. We wished to determine if a commercially available in-house ELISA would be applicable to sheep in our research facilities. Ultimately, an ELISA for detection of *Giardia* in sheep would result in improved accuracy and efficiency over microscopy, leading to rapid initiation of treatment and a reduced risk of zoonotic transmission to research staff. Fecal samples were collected from sheep ( $n = 93$ ) entering quarantine prior to enrollment in research studies. Samples were tested for *Giardia* by a combination of 6 different methods: fecal flotation at a reference laboratory, fecal flotation performed on site, *Giardia* ELISA developed for a reference laboratory, a commercially available EIA, an in-house rapid ELISA *Giardia* test, and a direct fluorescent antibody test. The prevalence of *Giardia* infection in sheep entering our facility was 11.8% (11 of 93 animals). Of the samples considered positive, only 3 of 11 were confirmed by all tests and 5 of 11 were positive only by microscopy. In-house fecal flotation found 8 samples which were positive on only 1 of 2 consecutive testing days. The commercial in-house rapid ELISA test typically used in dogs had a sensitivity of 0% for sheep giardiasis. Overall, the examined diagnostic methods had low sensitivities and positive predictive values when testing sheep feces, complicated by the intermittent shedding of the organism. Despite the limitations, microscopic analysis of repeat fecal samples remains the most accurate diagnostic method for giardiasis in sheep.

#### **P201 Isolation of Mouse Parvoviruses from Naturally Infected Mice and Vaccine Preparation against These Isolates**

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Mouse parvoviruses (MPVs) are small, single-stranded 5 Kb DNA viruses associated with asymptomatic, yet endemic infections in laboratory mice. Although these viruses may have little effect on the immune systems of most mouse stocks and strains, they may have deleterious effects in immunocompromised and genetically engineered mice (GEM). The frequent exchange of GEM among investigators from different institutions increases the likelihood of infection and multiplies possible MPV variants. Using our experience with papillomaviruses (PVs), we studied the phylogenetic trees of several MPV isolates using Clustal W analysis of viral genetic sequence data. Analysis allowed differentiation of endogenous from exogenous variants and revealed a separation into 2 different strains, which was used to postulate separate origins of various MPV diagnoses. We also constructed virus-like particles (VLPs) using the baculovirus expression system; tests to determine vaccine protection by VLPs of a single variant are underway. These findings demonstrate useful techniques for identifying important epidemiologic information regarding initial and subsequent discoveries of MPV exposure in murine colonies and promising methods of protecting precious GEM lines from infection.

#### **P202 Efficacy of Various Disinfectants for the Elimination of *Mycoplasma* on Environmental Work Surfaces**

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Prevention and elimination of *Mycoplasma* contaminants from cell cultures is a major concern for many investigators. *Mycoplasma* can remain viable in a dried out state for several days on work surfaces. PCR assays detect both viable and nonviable *Mycoplasma*. Culture procedures detect viable *Mycoplasma*, but are very time consuming and expensive. This report determines the efficacy of various disinfectants for the elimination of viable *Mycoplasma* when plated on petri dishes, dried, and then exposed to various disinfectants for 0, 10, 15, or 20 min using the ATP luminescence assay. Petri dishes were inoculated with 0.4 mL of a cell free *Mycoplasma* suspension or a cell culture suspension containing approximately  $1 \times 10^6$  *Mycoplasma* colony forming units of *M. arginini*/mL. *Mycoplasma* positive and negative control dried out cell cultures were maintained in a biosafety cabinet (BSC) to determine length of viability. After exposure to chemical disinfectants, samples were collected from the petri dishes using a sterile swab soaked in 1 mL of pyrogen and DNase/RNase free water and viability determined as per ATP assay. No viable *Mycoplasma* was recovered from the samples exposed to 0.5% or 3% hydrogen peroxide or chlorine dioxide and sodium

chlorite solutions at 10, 15, or 20 min. Low levels of viable *Mycoplasma* were detected in samples exposed to 70% ethanol or quaternary ammonium compound for 10 min. Positive control *Mycoplasma* in a dried out state remained viable for 7 to 10 d in a BSC. It was concluded that: 1) 0.5% or 3% hydrogen peroxide and chlorine dioxide or sodium chlorite solutions were more efficient than 70% ethanol or quaternary ammonium compound for eliminating viable *Mycoplasma* from environmental work surfaces; 2) the ATP luminescence assay can be used to measure the efficacy of various disinfectants on *Mycoplasma* viability and to determine the effectiveness of sanitation procedures.

#### **P203 Comparison of the Adenosine Triphosphate Luminescence Assay to the Polymerase Chain Reaction Assay for the Detection of *Mycoplasma* in Cell Cultures**

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*Mycoplasma* infects approximately 10% to 12% of all cell cultures and can significantly impact in vitro and in vivo studies. Culture procedures are time consuming (2 to 4 wk) and PCR assays are expensive. This report compares an ATP luminescence assay to a PCR kit for the detection of *Mycoplasma* in cell cultures. The ATP assay measures the increase in the level of ATP in relative light units (RLUs): < 1.0 = negative; 1.0 to 1.2 = retest; and > 1.2 = positive. PCR results were recorded as positive or negative. Over 283 cell culture passages collected from 50 different cell lines, over a 6-mo period including known positive and negative control samples, were assayed for *Mycoplasma* as per the instructions on each test kit. Results were analyzed using kappa statistics to assess agreement between the 2 methods. The distribution of low RLUs was examined to determine if the recommended cutoff points should be modified. From a total of 283 samples, 108 (38.2%) were ATP positive. In contrast, 49 of 283 (15.9%) samples were PCR positive. Sixty-eight of 283 (24%) were PCR negative and ATP positive. Using our revised cutoff points for the ATP RLUs: <1.6 = negative; 1.6 to 1.9 = retest; >1.9 = positive, 89 of 283 (31.5%) samples were ATP positive, and 51 of 283 (18.0%) were PCR negative and ATP positive. Some cell lines culture positive for *Mycoplasma* were negative using the PCR assay and positive by the ATP assay. We concluded that, 1) there was poor agreement between the 2 assays due to the increased sensitivity of the ATP assay and the failure of the PCR assay to detect some strains of *Mycoplasma*, 2) using the revised cutoff points for the ATP assay resulted in better agreement between the 2 assays, and 3) PCR assays detect both viable and nonviable *Mycoplasma* and may not detect some strains of *Mycoplasma* depending upon the PCR primers.

#### **P204 Effects of Administration of the IH901 on Muscular and Pulmonary Antioxidant Function after Eccentric Exercise**

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IH901 (20-O- $\beta$ -glucopyranosyl-20(S)-protopanaxadiol or compound K) is a final metabolite of ginseng in human intestine. The roles of IH901 administration in exhaustive exercise-induced oxidative damage in lung and muscle tissues are still not clear. Therefore, we studied whether IH901 administration can protect lung and skeletal muscle tissues and have beneficial effects on ameliorating exercise-induced oxidative stress in the lung and skeletal muscle of rats. This study investigated the interaction effect of IH901 and exercise training on oxidative stress markers and activities of antioxidant enzymes in soleus, gastrocnemius muscle, and lung of rats. Forty male rats were divided into the untrained IH901-deficient, trained IH901-deficient, trained IH901 low dose, trained IH901 middle dose, and trained IH901 high dose groups. The trained groups ran 35 min 2 d/wk for 8 wk. IH901 training interaction effect was significant on survey in skeletal muscles and lung tissue. The trained IH901-deficient group showed the highest thiobarbituric acid reactive substances, xanthine oxidase, myeloperoxidase, nitric oxide, plasma creatinine kinase, and lactate dehydrogenase; it showed the lowest glutathione peroxidase and superoxide dismutase activity in skeletal muscles and lung tissue. In contrast, thiobarbituric acid reactive substances, xanthine oxidase, myeloperoxidase, nitric oxide levels in trained IH901 groups were significantly lower than in the trained IH901-deficient group

( $P < 0.05$ ) and showed higher glutathione peroxidase, superoxide dismutase, and glutathione S-transferase levels than in the untrained IH901-deficient group and trained IH901-deficient group. These findings suggest that IH901 supplementation can prevent elevations of xanthine oxidase and myeloperoxidase activities in lung and muscle tissues and favorably influence the muscular and pulmonary antioxidant defense system after eccentric exercise.

#### **P205 In Vivo Distribution of Cy 5.5-Conjugated Thermally Cross-Linked Superparamagnetic Iron Oxide Nanoparticles in Mice**

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Among the many different types of nanoparticles currently being studied for applications in nanomedicine, superparamagnetic iron oxide nanoparticle is one of the emerging forms that can be used for in vivo imaging. Thermally cross-linked superparamagnetic iron oxide nanoparticles (TCL-SPION) are an antibiofouling polymer-coated SPION associated with use as a MR contrast agent for in vivo cancer imaging. After delivery, it is desirable for the nanoparticles to be removed or metabolized, ideally without any toxic side effects, from the body. The objective of this study was to investigate the distribution patterns of TCL-SPION in the body by fluorescence detection systems. Cy 5.5 conjugated TCL-SPION was injected intravenously to 5-wk-old male ICR mice (15 mg/kg BW in saline) 24 h before the imaging. After the whole body imaging, ex vivo optical images were taken in parallel in the organs such as liver, kidney, spleen, and lung. The fluorescence signal was detected after 30 min of injection in the liver and kidney, but not detected in the spleen until 4 h after the injection of TCL-SPION. In the lung, on the other hand, weak fluorescence signal was detected after 30 min, and disappeared after 2 h of injection. The distribution of TCL-SPION becomes visible in the reticuloendothelial system organs earlier than others. Further analysis is currently being carried out.

#### **P206 Occurrence of Mouse Parvovirus and Mouse Minute Virus in Mouse Colonies in Facilities in Minas Gerais, Brazil**

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Previous studies using indirect immunofluorescence assay (IFA) revealed only mouse minute virus (MMV) prevalence in Brazilian mouse colonies, once specific serologic assays to detect mouse parvovirus (MPV) were not available. We sought to diagnose MPV and MMV by PCR and serologic tests in mouse colonies breeding facilities in Minas Gerais. Plasmids containing parvovirus genomic DNA were obtained from the University of Arizona and were used to transform competent cells of *Escherichia coli* top 10, to be used as positive control in the PCR assays. Samples of spleen, mesenteric lymph nodes, and serums were obtained from animals in research facilities and/or public education institutions. One hundred and twenty-four mice from both sexes and different strains and ages belonging to 3 conventional facilities (A, B, and C) were evaluated by PCR assay. In facility A, twenty 5-wk-old mice and 56 with age ranging from 8 to 10 wk were analyzed. In the youngest group, only 10% (2) were positive for parvovirus; in the oldest group, 50% (28) were positive. In the last group, 21% (12) were positive for MPV, 38% (21) for MMV, and 9% (5) for both MPV/MMV. In facility B, 24 mice were evaluated, and 17% (4) were positive for MPV, 29% (7) for MMV, and 13% (3) for MPV/MMV. In facility C, 24 animals were also evaluated, and 33.3% (8) were positive for MPV, 37.5% (9) for MMV, and 16.7% (4) for MPV/MMV. The preliminary results demonstrate that MPV and MMV are highly prevalent in the investigated mouse colonies, and point to the necessity of investment in science and technology for production of animals with appropriate quality for scientific research.

#### **P207 Colonization of Abiotic Mice by Biofilm Bacteria**

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Our laboratory previously found that biofilm develops over time in automated watering system (AWS) manifolds and that purified water prevents biofilm growth in AWS manifolds for 1 y. However, the biologic significance of consuming bacterial biofilm from AWS racks is unknown. Consumption of biofilm may lead to subtle clinical effects in transgenic rodents with unknown immune status and/or subclinical effects that lead to increased variability of research results. To study these effects, we examined whether bacteria in AWS manifolds colonized abiotic mice. We provided groups of 3 abiotic outbred mice with water from an AWS rack with biofilm (M) or an AWS rack with purified water (B). Mice were housed in sterile cages in a laminar flow hood. Negative control groups received autoclaved water and were housed in the hood or germfree isolator. After 7 d mice were euthanized and the oral cavity, jejunum, cecum, colon, and liver were sampled for bacterial growth. Bacterial ATP analysis and culture of water were performed before and after the experiment. Samples were cultured aerobically and anaerobically on R2A and blood agar. Pre experiment water samples had substantial bacterial biofilm in M water and minimal to no biofilm in B water. Post experiment water had substantially increased bacterial ATP levels and significant growth on all 4 culture conditions for M and B. There was no evidence of bacterial contamination of autoclaved water. Of 6 mice exposed to autoclaved water, 5 had no growth from any organ. One mouse from the hood control group yielded a few colonies. All mice exposed to B water had significant growth from the cecum and colon on both agars. In addition 2 of these mice had significant growth from the jejunum on R2A agar. All mice exposed to M water had substantial growth from the cecum and colon, 2 mice had growth from the jejunum, and 1 mouse had growth from the oral cavity. The M biologic samples grew on R2A agar only. Results indicate that bacteria present in AWS manifolds can colonize the GI tracts of abiotic mice, with heaviest and most consistent colonization in the large intestine.

#### **P208 Characterization of Platelet Decline during Acute Simian Immunodeficiency Virus Infection in the Pig-Tailed Macaque (*Macaca nemestrina*)**

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HIV-associated dementia, with profound cognitive and motor impairment, develops in one-third of HIV-infected individuals. However, the pathogenesis of HIV CNS disease remains unclear. In our pig-tailed macaque model of HIV encephalitis, 70% of macaques dual inoculated with a neurovirulent clone (SIV/17E-Fr) and an immunosuppressive virus swarm (SIV/DeltaB670) develop CNS disease. We have identified platelet decline as a positive predictive marker for the development of neurologic disease in our SIV-infected pig-tailed macaque model and in HIV-infected patients. Platelets are emerging as key inflammatory cells in the pathogenesis of many infectious diseases such as viral hepatitis, malaria, and bacterial sepsis. To determine whether the decline in platelet numbers induced by SIV was associated with platelet activation, platelets and plasma were collected from SIV-infected and mock-infected pig-tailed macaques at 2-wk intervals for 84 d after inoculation. Platelet numbers were measured by a commercial veterinary clinical pathology lab, and platelet activation was assessed through measurement of the membrane-bound activation marker P-selectin using flow cytometry. After postmortem examination, brain tissue was evaluated for the development of SIV encephalitis by histopathology. Consistent with our previous observations, platelet number decreased by more than 100,000  $\mu$ L on days 7, 10, and 42 of acute SIV infection ( $P < 0.05$  by unpaired  $t$  test). This decline in platelet count was accompanied by a 10-fold increase in surface expression of P-selectin on days 10 and 42 postinfection ( $P < 0.05$  by unpaired  $t$  test), indicating that platelets were activated and implying that the observed platelet decline may be due to depletion secondary to an inflammatory role. We are now measuring soluble markers of platelet activation, including plasma serotonin, via ELISA, and examining the association between platelet activation and the development of encephalitis. These findings support a role for the activated platelet in the immune response to SIV.



## P209 Confounding Effects of Bedding on Rodent Obesity Models

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A variety of environmental factors can affect the outcomes of studies using laboratory rodents. From anecdotal observations we hypothesized that rats and mice used as diet-induced models of obesity (DIO) may ingest bedding and consequently impact metabolic study parameters. A prospective study was designed to determine the effects on certain physiologic parameters relevant to obesity models. Long-Evans (LE) and LE DIO rats were singly housed for 4 wk in cages containing alpha cellulose, corncob, and corncob with paper or wire mesh. C57BL/6NTac DIO mice or lean controls were also placed on the exact same 4 differing bedding types, with 1 substitution of hardwood chips in place of corncob with paper. DIOs were fed a 45% high fat chow while controls were fed standard chow ad libitum over a 14-d period. Blood glucose, body weight, and food consumption were measured. At necropsy stomachs were weighed and contents examined for evidence of ingested bedding. A least significant difference (LSD) mean comparison test (student *t* test) was used for analysis. *P* values were adjusted using Hochberg multiplicity adjustment to account for the many comparisons being performed and set at 5%. Results demonstrate all rats ingest their bedding but no single bedding type significantly affected fasting glucose levels ( $P > 0.0557$ ). The bedding used for mice, however, demonstrated an effect on baseline glucose levels for both DIOs and controls that correlated with ingestion of corncob bedding ( $P = 0.0147$ ). Weight gain and maintenance was not affected by the bedding used. These findings support the anecdotal observations made by staff and have led to a change of bedding used for obesity studies.

## P210 Evaluation of Vaporized Hydrogen Peroxide Disinfection for Prevention of Murine Norovirus Transmission

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Murine Norovirus (MNV) is environmentally stable and passed by fecal/oral route. Currently, disinfection of MNV employs chemicals that destroy sensitive equipment and machinery. Vaporized hydrogen peroxide (VHP) is a proven safe alternative for disinfection of sensitive equipment, although effects on MNV are unknown. This study evaluates the effectiveness of VHP disinfection on MNV transmission from known viral-infected surfaces. To determine the base MNV transmission under barrier isolator conditions, 40 MNV negative BALB/cj mice confirmed through sentinel serology and fecal MNV screening were pair-housed onto caging and in isolators that had previously housed confirmed MNV-infected animals. To determine efficacy of VHP on prevention of viral transmission, 40 confirmed MNV negative mice were pair-housed onto caging and in isolators that had previously housed MNV-infected animals and were VHP treated. Saline swabs of the caging before and after VHP were evaluated for virus by RT-PCR. Fecal samples were tested from each animal in each group at 7, 14, 21, 28, 35, and 42 d after exposure. Serology samples to evaluate antibodies to MNV were taken at 42 d after exposure for each group. Time points within groups were analyzed for infection risk by Kaplan-Meier curves with 95% confidence intervals. Risk of infection for time points between groups were analyzed with Fisher exact test, with significance of  $P < 0.05$ . Based on MNV fecal shedding, baseline exposure risk of infection was 80% by day 7 after MNV exposure increasing to a maximum 95% risk by 21 d. Risk of infection remained at 0% by 42 d after exposure to VHP-treated MNV caging. Infection risk between each group at each time point was significant ( $P < 0.0001$ ) with the non-VHP-treated group 99.9% more likely overall to shed virus compared to the VHP-treated group. Serology confirmed fecal results. In conclusion, this study verified VHP treatment of MNV contaminated caging effectively prevents transmission of MNV and VHP may be an effective alternative disinfection mechanism for MNV contaminated sensitive equipment.

## P211 Evaluation of Organ Weights, Serum Chemistry and Hematology Values, and Cecal and Nasopharyngeal Bacterial Cultures in the Gray Short-Tailed Opossum (*Monodelphis domestica*)

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*Monodelphis domestica* are currently used in genetic, developmental, oncology, and neurologic research. Very little is known about their natural flora or potential for pathogenic infectious disease. We aim to, 1) describe flora of clinically normal *Monodelphis domestica* to obtain a better understanding of potential pathogens in clinically abnormal animals and 2) improve existing comparative normal blood and organ weight values available to researchers. Clinically normal animals were assigned to 1 of 6 groups stratified by age (< 1 y, 1 to 2 y, and 2 to 3 y) and sex (male and female) for hematology and serum chemistry analysis ( $n = 5$  to 10 per group). Organ weights were measured on adult animals from the 1 to 2 y and 2 to 3 y groups ( $n = 30$ ). Data were analyzed using least squares analysis of variance with sex and age as fixed effects, significance defined as  $P < 0.05$ . Males have an 8% larger hemoglobin value and 20% higher phosphorus level compared to females. Females had significantly greater monocytes (36%) and eosinophils (67%) than males. In addition, a graded decrease in hemoglobin concentration was noted with increasing age. The group younger than 1 y of age had significantly higher levels of serum alkaline phosphatase and lower serum protein levels compared to older age groups. Females had significantly larger liver and kidney weights (on a per g body weight basis) compared to males. Nasopharyngeal and cecal bacterial cultures were taken from 20 clinically normal animals from the 2- to 3-y-old group. Nasopharyngeal culture revealed the predominant flora as *Streptococcus viridans* (50%), *Escherichia coli* (35%), and Coagulase negative *Staphylococcus* (25%). Cecal culture revealed the predominant flora as *Escherichia coli* (95%) and *Citrobacter* spp. (5%). The establishment of normal values for serum chemistry and hematology in *Monodelphis domestica* will aid researchers in comparisons and analysis of experimental values. The evaluation of flora of the nasopharynx and cecum in clinically normal animals will aid in diagnosis and evaluation of potential pathogens in clinically ill animals.

## P212 Cutaneous Phototoxicity in White Hanford Miniature Swine

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Phototoxicology safety testing in the miniature swine provides a unique opportunity for risk assessment because of demonstrated similarities to human cutaneous anatomy and physiology. In addition, the availability of several distinctive genetic origins provides a number of variants in hair and melanin pigment distribution. We present the results of the Hanford miniature swine skin irradiation study. The minimal erythema dose (threshold MED) from irradiation was first determined. Then six 5 cm<sup>2</sup> skin sites (control, vehicle, and 8-MOP at 0.0001%, 0.001%, 0.01%, and 0.1%) on each animal were scored at 10 timepoints (predose, 0, 1, 4, 24, 48, 72, 96, 120, and 144 h) following irradiation. One solar surrogate was used in these studies and consisted of a fluorescent fixture fitted with a combination of black light and sun lamp tubes. Exposures were monitored and controlled with a radiometer. The Hanford had a low UV dose threshold for response and the erythema that developed was readily observable. The MED determined for the Hanford was between 0.5 and 0.7 MED/h. The response developed within a few hours after exposure, and peaked at 48 h. In contrast, relatively mild erythema responses associated with the phototoxic drug 8-MOP developed first at 48 h and persisted for several days in the Hanford. The erythema dose response to 8-MOP was observed in the 2 highest concentrations and the intensity was minimal to moderate, but not severe. Microscopic evidence of a dose response was also observed as the level of 8-MOP increased. The Hanford Miniature Swine is a valid model for phototoxic effects.

## P213 Dermal Toxicology Application Area Changes as Compared to Theoretical Total Surface Area of Hanford Miniature Swine over 18 Weeks

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Dermal toxicology studies are frequently initiated in growing animal models. The test compound is generally applied on a dermal application area (DAA) that is frequently set at certain ratio to total surface area. Since the growth

during juvenile period can be significant, the DAA is likely to be impacted during the course of a chronic study. The purpose of this study was to, 1) determine if the ratio of the DAA to total body surface area (TBSA) will change proportionally over time with the growth of the miniature swine and 2) compare 3 TBSA formulas. The ratio DAA (cm<sup>2</sup> surface area) to TBSA was calculated from body weight at periodic intervals for 18 wk in 16 male and 16 female Hanford miniature swine (approximately 4-mo-old, averaging approximately 14 kg at initiation). Two midback 5 cm × 5 cm (25 cm<sup>2</sup>) DAAs, one located on each side of the spine, were used on each subject. The TBSA (m<sup>2</sup>) was calculated by the well recognized Spector formula ( $9.5 \times \text{BW}(\text{G})^{2/3}/10,000$ ) as well as the Brodie and Wachtel formulas. Using the Spector formula, the mean ratio DAA to TBSA for weeks 0 and 8 ( $n = 32$ ) for an application area was  $0.46 \pm 0.04\%$  and  $0.51 \pm 0.06\%$  (MEAN  $\pm$  SD), respectively. After week 8, subsequent periodic measurements of the ratio of TBSA to DAA remained steady suggesting proportional changes in growth of both DAA and TBSA. The correlation of the 3 TBSA formulas was greater than 0.99. Thus, comparable TBSA and DAA/TBSA ratio between the 3 formulas suggested the well recognized Spector method is a valid choice.

#### **P214 Physiologic Reference Ranges for Age-Matched, Wild Caught Black-Tailed Prairie Dogs (*Cynomys ludovicianus*)**

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The black-tailed prairie dog (*Cynomys ludovicianus*) is a member of the order Rodentia and the family Sciuridae and a keystone species in prairie ecology. They are also used as an animal model in laboratory investigations of gallbladder disease, *Clostridium difficile*, *Yersinia pestis*, and *Francisella tularensis* infections and most recently, *Orthopoxvirus* disease. Despite increasing numbers of prairie dogs in research and kept as pets, there is little data on their baseline physiology. To establish reference ranges our study used 18 wild caught black-tailed prairie dogs implanted with a transponder to record temperature and gross motor activity levels. This data was analyzed to establish circadian rhythms for activity and temperature. In addition, hematological and chemistry analysis was performed. Hematology analyses included counts of white blood cells (WBC), platelets (PLT), hematocrit (HCT), monocytes, granulocytes, lymphocytes, mean cell volume (MCV), red blood cells (RBC), hemoglobin (HGB), mean cellular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Analyzed blood chemistry parameters included alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium, chloride, creatinine, glucose, potassium, sodium, total bilirubin, total carbon dioxide, total protein, and blood urea nitrogen (BUN). Nine baseline measurements were used to establish the mean for each animal and these were compiled and analyzed to determine the reference ranges. The data from this study resulted in the establishment of more precise chemistry and hematology profiles as well as novel weight and core body temperature reference ranges and daily activity patterns for the black-tailed prairie dog. These results improve upon previous reports because they use multiple measurements from species and age-matched prairie dogs under controlled conditions, and will be useful to ecologists, scientists interested in using this animal model in lab investigations, and veterinarians caring for pet prairie dogs.

#### **P215 Response to Intraperitoneal Ketamine/Xylazine Anesthesia in Rats Is Unaffected by Short-Term Fasting**

MB Struck<sup>\*</sup>

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Ketamine/xylazine (KX) combined anesthesia is commonly used in rats. Researchers using this combination have reported an inconsistent anesthetic effect, with some rats showing an increased time between injection and onset of anesthesia, an inadequate anesthetic plane, or an inadequate anesthetic sleep time when compared with rats of the same strain, age, and weight. Although rodents are not routinely fasted prior to anesthetic events, there is anecdotal evidence that fasting rats prior to use of intraperitoneal ketamine/xylazine anesthesia results in a more rapid induction of anesthesia and a longer duration of surgical plane of anesthesia. This study tested the hypothesis that rats fasted for a short period (3 h) prior to induction of

intraperitoneal KX anesthesia would have a shorter induction time (IT), a longer total sleep time (TST), and a longer duration of loss of toe pinch reflex (TP) than fed rats. No previous research has been published comparing the effect of injectable anesthesia on fasted and unfasted rats. Twenty male Sprague-Dawley rats were housed in a reverse 12:12 light:dark cycle and used in a blinded, crossover experiment. KX anesthesia was administered to rats after a period of ad libitum feeding or a short period of fasting during the transition between the light and dark cycles. At doses of 50 mg/kg ketamine and 5 mg/kg xylazine, or 70 mg/kg ketamine and 7 mg/kg xylazine, there were no significant differences between groups in induction time, total sleep time, or toe pinch withdrawal reflex. We conclude that fasting prior to intraperitoneal KX anesthesia does not significantly affect induction time, total sleep time, or duration of time that a toe pinch withdrawal response remains negative, and that variability sometimes seen in reaction to KX anesthesia is unrelated to short-term fasting in rats.

#### **P216 Anemia in Hematopoietic Protein-1 Deficient Mice**

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Mammalian Hematopoietic protein-1 (Hem-1) is a hematopoietic cell-specific member of the HEM family of cytoplasmic scaffold proteins. Orthologs of Hem-1 in flies and worms are essential for cytoskeletal reorganization, embryonic cell migration, and morphogenesis. However, the *in vivo* functions of mammalian Hem-1 are not well understood. We previously identified a novel mouse strain containing a nonsense mutation in the Hem1 gene. Loss of Hem-1 resulted in severe lymphopenia, neutrophilia, and anemia. T cell development, activation, and adhesion were impaired, and neutrophils failed to migrate and phagocytose bacteria due to defective F-actin polymerization and cytoskeletal reorganization. Components of the Rac GTPase-controlled actin-regulatory WAVE complex were degraded in Hem-1 null neutrophils and T cells relative to wildtype cells. We sought to characterize a role for Hem-1 in erythrocytes by examining the mechanisms behind the regenerative anemia in Hem1-null mice. We hypothesized that loss of Hem-1 in erythrocytes results in disrupted WAVE complex formation, impaired F-actin polymerization, and malformed erythrocytes. Hem-1<sup>-/-</sup> erythrocytes exhibit significant structural abnormalities on peripheral blood smears, and increased osmotic fragility, relative to wildtype erythrocytes. Hem-1<sup>-/-</sup> erythrocytes also have a shortened lifespan compared to wildtype erythrocytes when transferred into lymphocyte-deficient Rag2<sup>-/-</sup> Gamma chain<sup>-/-</sup> hosts. Immunoblot analyses of erythrocyte lysates indicate that components of the WAVE complex are expressed in wildtype and Hem-1<sup>-/-</sup> erythrocytes, although the protein levels appear decreased in the absence of Hem-1. These results suggest that Hem-1 regulates erythrocyte morphology, perhaps by regulating the WAVE complex and reorganization of the actin cytoskeleton. Further research is needed to determine whether Hem-1 modulates other protein components of the erythrocyte cytoskeleton (for example, spectrin or adducin), and how these interactions may also contribute to anemia.

#### **P217 Comparison of Ketamine/Xylazine and Ketamine/Dexmedetomidine Anesthesia and Their Effects on Electrocardiograms in Rice Rat (*Oryzomys palustris*)**

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Rice rats have a high degree of susceptibility to destructive periodontal disease, thus making them a good model for periodontitis. When the experimental design requires extraction, the method of anesthesia is challenging in this model since gaseous anesthesia cannot be used easily. The classic par-enteral general anesthesia, a combination of ketamine and xylazine, can be used; however, prolonged recovery time was encountered in the rice rats. We sought to determine the effects of ketamine-dexmedetomidine anesthesia on electrocardiograms (ECG) in comparison with ketamine-xylazine anesthesia. The animals were divided in 3 groups to receive ketamine-xylazine (75 and 10 mg/kg, group A,  $n = 5$ ), ketamine-dexmedetomidine (41 and 0.136 mg/kg, group B,  $n = 4$ ), or ketamine-dexmedetomidine (71 and 0.235 mg/kg, group C,  $n = 3$ ) administered intraperitoneally. Once a surgical plane was reached, after 20 min, animals in group A were given yohimbine (4 mg/kg), whereas animals in group B and C received atipamezole (1.25 and 2.5 mg/

kg, respectively). Parameters measured included time to onset of anesthesia, time to recovery from anesthesia, respiratory rate, and ECG biomarkers. The time to onset of anesthesia was not significantly different between each group. Forty percent of animals in group A (2 of 5) and 25% of animals in group B (1 of 4) did not maintain a surgical plane of anesthesia of 20 min. In group B, the stage III of anesthesia was maintained at higher respiratory rate (RR) but lower heart rate (HR) when compared to animals in group A. The RR and HR were slower at higher dose of ketamine-dexmedetomidine. Atrial premature depolarization was observed in animals in group A (4 of 5). No abnormal cardiac rhythm was observed in animals in group B and C. The recovery time to withdrawal reflex after injection of reversal agent in group B (2.0 min) and C (2.2 min) was significantly shorter than group A (4.2 min;  $P < 0.05$ ). The recovery time of animals in group B (6.4 min) was significantly shorter when compared to group A (21.1 min;  $P < 0.05$ ). The results suggested that intraperitoneal ketamine-dexmedetomidine is a preferable anesthetic in rice rats for parenteral anesthesia.

#### **P218 Development of a Fecal PCR Assay for Detection of *Helicobacter ganmani* Infections in Laboratory Rodents**

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Laboratory rodents may be infected with several species of *Helicobacter* which vary widely in pathogenicity and effects on biomedical research. *Helicobacter ganmani* is an anaerobic urease negative extragastric *Helicobacter* that is phylogenetically close to *H. rodentium* and infects both mice and rats with unknown prevalence or impact on research. The only published molecular diagnostic test for *H. ganmani* is a seminested PCR assay amplifying a region of the 16S to 23S internal spacer region (ISR) designed to survey pediatric liver biopsies for the presence of *H. ganmani*. Seminested assays are problematic for use in routine rodent diagnostics because of the potential increased incidence of false positive results and the increased expense of performing 2 amplifications. The objective of this study was to develop a sensitive and specific PCR assay for *H. ganmani* and adapt the assay to a multiplex format that will allow specific identification of 6 *Helicobacter* species found in rodents: *H. hepaticus*, *H. bilis*, *H. rodentium*, *H. trogontum*, *H. typhlonius*, and *H. ganmani*. Unique regions of the 16S rRNA gene were identified and *H. ganmani*-specific primers were designed that amplified a 79 bp fragment. The specificity of the *H. ganmani* primers was evaluated against DNA from *H. hepaticus*, *H. bilis*, *H. rodentium*, *H. trogontum*, *H. typhlonius*, and *H. ganmani*. The results of these trials showed that the *H. ganmani* primer set amplified only *H. ganmani* DNA as confirmed by sequence analysis. The *H. ganmani* PCR assay was successfully included in a multiplex PCR assay for simultaneous detection of multiple rodent *Helicobacter* species in rodent feces. In summary, the newly developed PCR assay is a valuable tool for *Helicobacter* detection and speciation in laboratory rodents.

#### **P219 Dietary Treatment Eradicated *Helicobacter* spp. from Young Mice Deficient in Natural Killer Cells**

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Several *Helicobacter* species are proven research confounders. Despite this, prevalence in rodent research colonies remains high. A dietary treatment similar to that used for human infections could prove a cost and time effective strategy to eradicate *Helicobacter* spp. from affected rodent colonies. Recently, a commercially available 4 drug combination, nutritionally complete dietary tablet containing amoxicillin, metronidazole, clarithromycin, and omeprazole has shown promise in eradicating *Helicobacter* spp. from multiple mutant rat strains and 1 strain of immune competent mice, while its effectiveness in immune deficient mice is unknown. This study evaluated the efficacy of this 4 drug combination in eradicating *Helicobacter* spp. from B6.129-Cd1tm1Gru mice deficient in natural killer (NK) cells. Twenty young mice aged 8 to 12 wk and 12 older mice aged 6 mo or more that were naturally infected with *H. hepaticus* with or without *H. rodentium* were singly housed and fed either control or treatment diet for 8 wk. Fecal samples were collected from each mouse every 2 wk and evaluated by polymerase chain reaction for *Helicobacter* spp. Animals were followed for an additional 4 wk after termination of treatment. Tissues were evaluated by gross necropsy and gastrointestinal and liver samples were examined by histopathology. All

mice in the young treatment group ceased shedding *Helicobacter* spp. after 2 wk of treatment and remained negative throughout the study. Two male mice from the adult treatment group intermittently shed *Helicobacter* spp. after 6 wk but remained negative at the end of the treatment. Interestingly, all treated mice had significantly larger ceca than untreated controls. These findings demonstrate that the diet containing amoxicillin, metronidazole, clarithromycin, and omeprazole rapidly eradicated *H. hepaticus* and *H. rodentium* from young NK deficient mice, but was less effective in established infections in these older mice. These results suggest that dietary treatment can be effective for *Helicobacter* spp. eradication in young mice.

#### **P220 Comparative Bone Anatomy of Commonly Used Laboratory Animals Using Micro CT: Implications for Drug Discovery**

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New technology has improved the science of bone comparative anatomy by enabling a hierarchical evaluation across species from the gross organ to the tissue and cellular levels. We used micro CT to evaluate differences in the gross anatomy and geometry of the proximal femur, lumbar vertebrae, and mandible in commonly used preclinical species including mice, rats, rabbits, dogs, and NHPs. The technology also provided quantitative measures of internal bone structure and bone mineral density. Specimens were scanned on a micro CT system. The results demonstrated a large variation in the gross anatomy and structural properties of the proximal femur between compared species. Cortical bone mineral density (BMD) was slightly higher in modeling species such as mice, rats, and rabbits relative to dogs and NHPs, perhaps due to Haversian system and intracortical remodeling in the latter species. We also noticed that remodeling species have larger bone marrow volume, which could be a consequence of bone resorption at endocortical envelopes. Intuitively, cortical thickness parameter increases with size and weight of the species in order to meet mechanical demands of the skeleton. At the femoral head, the ratio between bone volume and tissue volume was the highest in mice and lowest in NHPs. Trabecular number appeared to follow the same pattern, being the highest in mice and lowest in NHPs. Since trabecular thickness was similar across all species we concluded that difference in trabecular separation and connectivity between species is due to difference in trabecular number, rather than thickness and that trabecular connectivity could be the main determinant of bone strength at this skeletal site. There are differences across species in the gross anatomy of the femoral neck (overall size, length, and morphology). These morphologic and structural differences reflect mechanical and metabolic demands that are species specific. Based on the results, scientists should be able to better select the preclinical models that feature skeletal characteristics relevant for their research while allowing for improved translation to the clinical setting.

#### **P221 Coinfection with the Nematode *Heligmosomoides polygyrus* Reduces Intestinal Metaplasia and Dysplasia in the INS-GAS Mouse Model of *Helicobacter pylori*-Associated Gastric Cancer**

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Transgenic INS-GAS mice (Tg(Ins1-GAS)1Sbr) develop hypergastrinemia and thus are predisposed to gastric hyperplasia, atrophy, and gastrointestinal neoplasia (GIN). Male INS-GAS mice infected with *Helicobacter pylori* develop gastric carcinomas by 7 mo postinfection (mpi). Prior studies have shown that coinfection with the murine nematode *Heligmosomoides polygyrus* stimulated an antiinflammatory Th2 response that ameliorated gastric atrophy associated with *H. felis* infection in C57BL/6 mice. We evaluated 9 male *Helicobacter*-free INS-GAS mice orally dosed with 200 third stage *H. polygyrus* larvae at 6 wk of age followed in 1 mo by 1 × 10<sup>8</sup> colony forming units of *H. pylori* SS1. Controls included 9 uninfected mice, 12 mice infected with *H. pylori* alone, and 13 mice infected with *H. polygyrus* alone. Persistent infection with *H. pylori* was confirmed by quantitative PCR and with *H. polygyrus* by elevated serum IgE levels. Mice were necropsied at 5 mo postinfection for histopathology. *H. pylori*-induced gastritis was most severe in the corpus and was associated with inflammation, epithelial defects, atrophy, hyperplasia, dysplasia, and intestinal metaplasia ( $P < 0.05$ ).



Coinfection with the nematode *H. polygyrus* significantly reduced intestinal metaplasia ( $P < 0.02$ ) and dysplasia ( $P < 0.04$ ). Intestinal metaplasia and dysplasia are important precursor lesions to GIN which developed in 1 of 12 mice infected with *H. pylori* alone. These results are consistent with our previous report using the *H. felis*/*H. polygyrus* mouse model and human epidemiology studies that demonstrated an association between reduced risk for gastric cancer in *H. pylori*-infected adults when, as children, they were infected with a variety of gastrointestinal parasites. Importantly, the *Helicobacter*-infected INS-GAS mouse can be used to model parasitic and other implicated cofactors that may inhibit or promote gastric cancer in humans.

### **P222 Influence of Paradoxical Sleep Deprivation on Memory Consolidation Using Ethical Discriminative Avoidance Task in Young Adult Rats**

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Numerous studies have demonstrated physiologic alterations induced by sleep deprivation in laboratory animals. A myriad of evidence has suggested that sleep plays an important role in the memory/learning processes. In this context, physiologic modifications could be involved in cognitive performance levels verified across life span. We aimed to investigate whether age would be able to produce modifications in acquisition and/or retention induced by paradoxical sleep deprivation (PSD) in rats. Male Wistar rats (30, 45, 60, 75, or 90-d-old) were submitted to the training session in the plus-maze discriminative avoidance task (PM-DAT). PM-DAT is an animal model that evaluates concomitantly learning/memory, anxiety, and motor activity in rodents without applying any painful stimuli. Immediately after the first session, half of the group was returned to their home cage (CTRL) while the other half was paradoxical sleep deprived for 96 h. The rats were tested in PM-DAT 48 h after the PSD period. Our results showed that in the training session no differences were observed concerning acquisition levels, anxiety-like behavior, or motor function. When memory was evaluated (test session), only 90-d-old rats submitted to post training PSD-96 h presented impairment in retention of the discriminative avoidance task. In addition, such impairment was not accompanied by alterations in anxiety or locomotive activity. Our data suggest that although post training PSD had deleterious effects on memory, these effects were counteracted by age. Thus, the brain plasticity verified during development could prevent PSD-induced amnesia. In conclusion, PSD-induce amnesia can be critically influenced by age in rats.

### **P223 Practical Clinical Chemistry for Rodents: Dilution Effects**

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Clinical chemistry is an essential diagnostic tool in veterinary and medical clinical practice, and is an important analytic tool in many areas of research, drug assessment, and development. In research involving mice or other small animals, small blood volumes limit the practical utility of clinical chemistry analyses on serum or plasma. Terminal blood collections of approximately 1 mL blood from a 20 to 30 g mouse typically yield less than 400  $\mu$ L of serum or plasma. Many mouse serum or plasma specimens submitted to our laboratory are less than 100  $\mu$ L total, and are insufficient for chemistry tests with our automated analyzer. The aim of this study was to determine what chemistry analyses yield reproducible results relevant to results from undiluted specimens. Dilutions (with 0.7% NaCl) and tests were performed on terminal blood collections (> 1 mL) obtained via cardiocentesis, immediately after CO<sub>2</sub> euthanasia, from 36 adult mice weighing more than 25 g that were destined for euthanasia under IACUC approved protocols. Two fold, 3-fold, and 5-fold dilutions of whole blood (prediluted specimens) and of plasma (post diluted specimens) were compared to results from nondiluted specimens. Calculated results from 2 times and 3 times post diluted specimens correlated well (> 90% similarity) with results from nondiluted specimens. Calculated results from prediluted specimens and from 5 times post diluted specimen did not correlate as well (< 90% similarity) with results from nondiluted specimens. In conclusion, when specimen dilution will permit essential testing on small, valuable, or fewer animals, all specimen dilutions should

be performed identically throughout the study, and the validity of the prioritized selected tests should be evaluated for the dilution method before undertaking the study.

### **P224 Increased Collagen Type VI Content in the Hearts of Cardiomyopathic Syrian Hamsters (J2N Strain)**

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Cardiomyopathy is a serious heart disease with various causes, which makes it essential to clarify its etiology. Our previous studies using mouse and monkey models suggested an increase of collagen type VI in the cardiomyopathy. To confirm if the collagen type VI commonly increases in cardiomyopathy of a variety of animal species, we compared heart protein profiles between normal and cardiomyopathic Syrian hamsters. We used normal (J2N-n strain) and cardiomyopathic (J2N-k strain) hamsters at 4 wk (no clinical symptoms in both J2N-n and J2N-k) and 4 mo of age (heart symptoms in J2N-k but not in J2N-n). Heart proteins from the 4 groups, 3 males per group, were extracted sequentially from left ventricles into soluble, less soluble, and least soluble fractions according to their solubility. These fractions were separated by SDS-PAGE. We focused on proteins of the least soluble fractions because our previous studies found out that subunits of the collagen type VI were present in the fraction. On staining the gels, bands of collagen type VI  $\alpha$ 3-subunits (approximately 200 kD) tended to be denser in the cardiomyopathic hearts at 4 mo of age than in those of the other 3 groups. Western blot analyses confirmed the increase in collagen type VI  $\alpha$ 1- and  $\alpha$ 2-subunits in 4-mo-old J2N-k hearts in comparison with the other 4 groups. These results indicate that no difference was found in the amounts of heart collagen type VI subunits in young hamsters of both strains without heart symptoms and that the difference became evident in 4-mo-old J2N-k hamsters with enlarged hearts. These results confirm that collagen type VI increases during the course of cardiomyopathy in 3 animal species. The collagen type VI would be a good target for therapy and diagnostics of cardiomyopathy.

### **P225 Pharmacokinetics of Buprenorphine in Mice Administered Subcutaneously, Intravenously, Orally by Gavage, and Orally by Voluntary Ingestion**

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Buprenorphine is the most commonly used drug for peri-operative pain relief in laboratory rodents. It is commercially available in formulations for parenteral and sublingual administration. We examined buprenorphine concentrations in the circulation of mice following administration subcutaneously, intravenously, orally by gavage, and orally by voluntary ingestion of nut paste containing the drug. The aim was to investigate the efficacy of self-administered pain relief treatment in mice, and to apply this system as default throughout the institution's experimental sections in which rodents are subjected to invasive surgical procedures. Buprenorphine mixed in a worldwide commercially available popular sticky nut paste for human consumption, readily consumed by mice and rats, resulted in long-lasting high buprenorphine concentrations in the circulation (AUC<sub>24h</sub>: 120 ng $\times$ h/mL), as did oral gavage administration (AUC<sub>24h</sub>: 130 ng $\times$ h/mL). By contrast buprenorphine administered intravenously and subcutaneously remained in the circulation for a shorter time (AUC<sub>24h</sub>: 26 ng $\times$ h/mL and 54 ng $\times$ h/mL, respectively). This marked difference is most likely due to the higher dose used for oral administration (0.4 mg/kg bw, as compared to 0.05 mg/kg bw for parenteral routes), which is regarded necessary for sufficient analgesic effect, and for saturation of the enzymes responsible for first pass liver metabolism of buprenorphine. In conclusion, the bioavailability of buprenorphine after voluntary ingestion in sticky nut paste was found satisfactory. Therefore, it constitutes a practical way to noninvasively provide laboratory rodents with efficient pain relief when the animals are subjected to invasive recovery surgery.

### **P226 A Refined Blood Collection Technique in Cynomolgus Macaques (*Macaca fascicularis*) for Pharmacokinetic Studies**

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Historically, collecting serial blood samples for pharmacokinetic (pk) studies in cynomolgus macaques has been performed by repeated venipuncture of either the femoral or cephalic veins or by using subcutaneous vascular access ports. These methods can be done successfully with minimal complications, but generally required prolonged chair restraint during the early part of the study and/or repeated handling of the monkeys in moving them back and forth to the chairs. A refinement of these procedures, designed to reduce or eliminate the presumed discomfort of repeated venipunctures and/or the stress associated with the accompanying restraint procedures, was desirable at our institution. In pursuit of moving toward an automated blood sampling methodology in macaques, we found several procedural hurdles when dealing with cynomolgus macaques on a tether being used for pk studies that required innovative solutions. Many of these challenges involved safety concerns, animal handling, animal acclimation to devices and procedures, labor, efficiency, and specialized equipment. Vessel selection included either the femoral vein or artery due to our experience with higher patency rates over the jugular vein. Cage modifications and jacket customizations were required to allow for dosing and easy access to vascular access ports. After many months of development, we have devised an efficient system and process that required numerous innovations to solve logistical, mechanical, animal behavioral, and efficiency dilemmas. In addition, this refined blood collection method improves both the science and animal welfare by being less stressful for the monkeys, as evidenced by subjective observation during their collection time points where normal behavior was witnessed (for example, eating, drinking, and interactive behavior) compared to our standard technique. An additional outcome of this refinement has been the positive response of the technicians to this minimally invasive sampling technique and an increased level of safety due to a much reduced requirement for repeated handling of the monkeys and biohazardous sharps material.

#### **P227 Gait Analysis and Behavioral Pain in Two Rodent Models of Osteoarthritis**

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We evaluated gait pattern recorded on the automated gait analysis system and pain behavior in 2 different rat models of osteoarthritis (OA). Twenty-two male Sprague-Dawley rats weighing  $200 \pm 25$  g were studied. Animals were trained on the gait analysis system runway to traverse the corridor uninterrupted. Mechanical allodynia was assessed by measurement of withdrawal thresholds in response to application of von Frey filaments. Prior to the knee intervention, data was collected to obtain baseline values. One group of rats ( $n = 8$ ) underwent surgical anterior cruciate ligament transection with partial medial meniscectomy (ACLT+pMMx) to mimic a joint instability model and another ( $n = 8$ ) received an intra-articular injection of monoiodoacetate (MIA) (3 mg/30  $\mu$ L), an inflammatory pain model. After recuperation, tests were performed for 4 consecutive weeks. After the behavioral measurement period, rats were sacrificed. Both knee joints were collected for histologic assessments. A tendency towards stabilization in the surgical model was observed with parameters (swing duration, swing speed, and stance phase/stepcycle ratio) returning near the baseline values. Conversely, the MIA model showed significant changes remained in the injured limb compared to the contralateral limb in the swing phase duration ( $P < 0.02$ ) and the swing speed ( $P < 0.02$ ) demonstrating clear evidence of a limping gait. With von Frey filaments, mechanical sensitization was observed in the ipsilateral limb of the MIA model only ( $P < 0.0001$ ). Histologic evaluations revealed severe cartilage loss in the MIA model and only minor changes in the ACLT+pMMx model, confirming articular lesions in both models. In conclusion, clearer behavioral nociceptive responses related to gait parameters of the osteoarthritic limb were seen with the MIA model suggesting that it may be a better model for the rapid evaluation (1 mo) of therapeutic strategies for joint pain palliation with respect to the length of the study.

#### **P228 Parameters Expected in Wistar Rats Using Two-Way Anesthetics**

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During the experimental protocols to minimize pain and stress, the animals are often anesthetized using 1 of 2 anesthetics, isoflurane or ketamine/xylazine.

The administration of anaesthetics can cause disruption to thermo-regulatory mechanisms as well as the cardiovascular, respiratory, and central nervous systems. We hypothesize that taking into account the type of anaesthetic used can determine the effect on biochemical parameters (listed below). We used sixty 2-mo-old male Wistar outbred rats. Whole blood samples (arterial or venous) were obtained using heparinized needle and syringe in anaerobiosis. Puncture of the vessels of the tail was performed under inhalation anaesthesia, isoflurane (group 1), or intraperitoneal, ketamine/xylazine (group 2). To assess their impact, we carried out the dosage of the following parameters: acid-base status (pH, oxygen pressure, carbon dioxide pressure, bicarbonate, base excess, and oxygen saturation), hematocrit, hemoglobin, ions (sodium, potassium, calcium, and chlorine), glucose, and lactate. We also took the age and sex of the Wistar strain used into account when measuring these parameters. For both arterial and venous blood samples, we observed in group 2 compared to group 1, a decrease in pH and oxygen pressure, and therefore, the oxygen saturation, and an increase in carbon dioxide pressure and glucose. Both groups showed a decrease in sodium and an increase in lactate compared to the internationally accepted protocols. Furthermore, the data we obtained will serve as a database to be used in future protocols, which will help us reduce the number of animals in the control groups, allowing us to meet the reduction ideal of the 3Rs. The increase of glucose and lactate and the decreased pH and oxygen pressure indicate a state of stress that needs to be confirmed with further studies. It is important, when planning an experimental protocol, to choose an anaesthetic according to the positive or negative effects on the animal. Therefore, the effects of anaesthetics should be considered when interpreting the biochemical parameters.

#### **P229 Radiation-Induced Sarcomas following Single-Dose Irradiation of C3Hf/Sed Mice with Dose-Response Relations**

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Concern for the risk of radiation-induced cancer is rising with the increase in the number of cancer patients surviving long term, especially younger patients. This study examines the risk of locally and systemically radiation-induced cancer, following single dose local irradiation in the inbred young mice. A total of 326 (164 male, 162 female) 5-d-old C3Hf/Sed mice were used. Their legs were given a single radiation dose ranging from 0 to 22 Gy. All mice were observed for 18 mo after irradiation, and then necropsied. All grossly observed lesions were studied histopathologically. Cancer developed in the irradiated area at rates significantly above background control levels over the dose range of 6 Gy to 22 Gy, with an incidence ranging from 3% to 30.4% (average = 13.7%). No cancers developed in the 0 Gy group (55 mice) in the lower extremities area. The incidence of malignancy increased with dose up to about 14 Gy. A total of 38 cases of cancer were detected in the irradiated area, of which 37 were sarcomas (32 fibrosarcomas, 2 rhabdomyosarcomas, 2 osteosarcomas, and 1 hemangiosarcoma), and 1 was a squamous cell carcinoma. The incidence of spontaneous tumors in organs or tissues outside the irradiated field was 27.3% in the mice in the 0 Gy group and 13.3% to 35.3% in the 6 Gy to 22 Gy groups (average: 22.1%). The most common spontaneous tumor in males was liver hepatocellular carcinoma (33 of 36). In females, ovarian tumors (15 of 39) were the most common, followed by subcutaneous sarcomas (14 of 39), and mammary gland tumors (6 of 39). No significant differences in incidence outside the irradiated field were found between the 0 Gy group and the individual groups receiving 6 Gy to 22 Gy. In conclusion, single-dose irradiation induced sarcomas in 5-d-old C3Hf/Sed mice in the area of local irradiation, but did not increase the incidence of tumors outside the irradiated field. The incidence of radiation-induced sarcoma increases with dose up to a maximum at 14 Gy, and then it decreased with increasing dose up to 22 Gy. Using a relative risk model, these results provide data for estimation of the risk of radiation-associated cancer in young patients, and the dose tumor incidence relationship.

#### **P230 Compound Solubility with Fascioliscide Activity: Efficacy Evaluation In Vitro and in Rabbits Experimentally Infected with *Fasciola hepatica***

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Compound Alpha (Ca) is a benzimidazole derivative with great fascioliscide oral activity; its solubilisation is an alternative to get an injectable formulation. We assumed Ca administered intramuscularly will have a similar or higher effect than oral administration. We wanted to evaluate Ca solubility and determine fascioliscide efficacy *in vivo* and in rabbit experimentally infected. Ca was synthesized and vehicles were obtained from a commercial source. Triple solubility essays were performed and measured with a high resolution liquid chromatographer (HPLC). We found that beta cyclodextrin and hydroxypropyl-beta-cyclodextrin at 2 mg/mL solubilized at 0.051% and 1.018%, respectively; the hydroxypropyl-beta-cyclodextrin at 5.8 mg/mL was obtained at 3.95%, hydroxypropyl-beta-cyclodextrin and methanol at 10.76%, propylene glycol (PG) at 39.048%, glycerol formal (GF) at 100%, and GF with PG and water (3:2:4 and 3:2:5) at 100%. The test *in vitro* with immature fasciolas was done in conformity to Rivera's et al, 2004 description. The evaluation *in vitro* proved the efficacy of Ca with GF and vehicle combination at 24 h at 100%. For *in vivo* test, rabbits infected with 90 metacercarias were divided into 3 groups of 6 animals each, to which we administered 1 and 2 mg/kg/IM concentration of 6.52 mg/kg; the third group was a control group (saline solution). We took blood samples at 0, 15, 30, 60, and 120 min and at 4, 8, and 16 h after the treatment to look for Ca and its sulphoxide and sulphone metabolites using HPLC. The efficacy was determined based on the formula proposed in presence of fasciolas in the group treated versus the control group. In the statistical essay we did a variance analysis prior data transformation of Box and Cox. In rabbits we had an efficacy of 16.67% and 68.4%, respectively; in serum both Ca and its metabolites were not found. It is concluded that Ca was solubilized with high efficacy with fascioliscide *in vitro*, but in rabbits the efficacy was moderate.

### **P231 The MGH-MHC-Defined Miniature Pig as a Model for Graft versus Host Disease in Hematopoietic Cell Transplant Studies**

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MGH MHC-defined miniature swine provide a preclinical model with responses to hematopoietic cell transplantation (HCT) that closely resemble those in humans, making this model well-suited to preclinical studies involving graft versus host disease (GVHD). HCT between MHC matched or mismatched animals or haploidentical transplants can be performed to mimic clinical HCT scenarios. With standard myeloablative conditioning, HCT across MHC barriers is most often fatal, with animals developing severe grade III to IV GVHD involving the gastrointestinal tract (GI), liver, and skin. The clinical course, clinical pathology, and histopathology of pigs undergoing GVHD resembles what is observed in humans, where GVHD continues to be a major complication of HCT. We have developed a novel protocol with reduced incidence of GVHD. Recipients receive a very large (“mega”) HCT dose of  $150 \times 10^9$  cells/kg and undergo nonmyeloablative conditioning consisting of CD3-immunotoxin, 100 cGy of irradiation and cyclosporine for 45 d. This protocol has induced reliable stable mixed chimerism with minimal GVHD, presumably by harnessing strong immunomodulatory mechanisms. However, some animals still exhibit GVHD following HCT or DL, similar to humans. We are now studying the effector mechanisms responsible for the development of GVHD and/or development of tolerance in this miniature swine model. Unlike rodent models, these miniature swine provide an opportunity to perform extended longitudinal studies on individual animals, since multiple tissue biopsies can be taken without the need to sacrifice the animal. Given the similarities of GVHD in pigs and humans, we hope that the results of these studies will be applicable to the development of new strategies to ameliorate the results of clinical HCT across MHC barriers.

### **P232 Preparation of Antigens for Serological Screening of Simian Foamy Virus, Simian Cytomegalovirus, Simian Varicella Virus, and Simian Virus-40 in Macaques**

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The current minimally accepted definition for specific pathogen-free (SPF) laboratory macaques is an animal that is free from infection by simian immunodeficiency virus (SIV), simian type D retrovirus (SRV), simian T-cell lymphotropic virus (STLV), and B virus. However, macaques are susceptible to infection by a wide variety of other adventitious viruses that can impact biomedical research studies including simian foamy virus (SFV), simian cytomegalovirus (SCMV), simian varicella virus (SVV), and simian virus-40 (SV-40). These viruses are thought to be widespread within domestic macaque colonies; however, serodiagnostic screening is rarely performed so the true prevalence of these viruses is largely unknown. To address this issue, we developed antigens for detection of antibodies against SFV, SCMV, SVV, and SV-40. Mammalian (human and/or simian) cell lines were infected with these viruses and infected cells were harvested by low speed centrifugation. The virus preparations were pelleted by high speed ultracentrifugation and then purified by density gradient ultracentrifugation. The purified virus antigens were then coupled to polystyrene microspheres (beads) and used to develop a multiplexed fluorometric immunoassay (MFIA) for simultaneous detection of antibodies against all of these viruses in macaque sera. The prevalence of SFV, SCMV, SVV, and SV-40 in domestic laboratory macaque populations was determined by testing 627 serum samples from 11 different macaque colonies by MFIA and found to be 63%, 71%, 0%, and 40%, respectively. These results show that the prevalence of SFV, SCMV, and SV-40 is very high within macaque colonies and that SVV, which was not detected in this limited serosurvey, is not widely spread within the domestic macaque colonies that were surveyed.

### **P233 Dark-Phase Light Contamination in Laboratory Animal Facilities Induces Circadian Disruption in Nude Rat Metabolism and Promotes Diabetes and Human Cancer**

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Dark-phase light contamination, as sometimes occurs in laboratory animal facilities, may result in chronobiologic rhythm disruptions that impact the health and wellbeing of animals and influence the outcome of scientific investigations. Light at night, via its ability to induce suppression of circadian pineal melatonin production associated with insulin and glucose homeostasis, may account for the significant increase in type 2 diabetes (T2D) and cancer in rotating night shift workers. Here, in conjunction with our recent human cancer studies, we examined whether suppression of the nocturnal melatonin signal disrupts temporal coordination of mammalian central and peripheral clock gene mechanisms regulating animal metabolism that predispose to T2D. Female nude rats bearing tissue-isolated MCF-7 human breast cancer xenografts were maintained on either a control (C) 12L(141.5  $\mu$ W/cm<sup>2</sup>):12D or experimental (E) 12L:12D-(0.08  $\mu$ W/cm<sup>2</sup>) light/dark cycle (lights on at 0600). Measurements of tumor tissue clock (BMAL1, CLOCK, PER1, CRY1) and clock-associated (WEE1 and cMYC) gene mRNA expression, arterial plasma melatonin, total fatty acid (TFA), triglycerides (TGA), free fatty acids (FFA), phospholipids (PL), cholesterol esters (CE), glucose, lactic acid, and corticosterone levels were made every 4 h over a 24-h period ( $n = 6$  per time point). Normal circadian expression of tumor clock gene mRNAs in C was disrupted in E. Plasma melatonin levels in C were high in the dark phase ( $108.8 \pm 6.5$  pg/mL), low ( $1.0 \pm 0.2$  pg/mL) in the light phase and low throughout the 24-h period in E. Diurnal plasma TFA and lipid fraction levels (TGA>FFA>PL>CE), glucose, lactic acid, and corticosterone levels in C (high, night; low, day) were phase-delayed 4 to 8 h and elevated in E ( $P < 0.05$ ). These findings demonstrate that integrated circadian rhythms of clock gene expression and metabolism *in vivo* can be disrupted by light at night via melatonin suppression to promote T2D and human breast cancer.



### P234 Serial Blood Sampling for Pharmacokinetic Studies in Mice

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There is an increased emphasis on target validation and development of efficacy models in drug discovery to address the Phase II attrition of clinical candidates in the pharmaceutical drug development cycle. The primary animal efficacy model used in oncology based drug discovery studies is the mouse. Initial pharmacokinetic studies of discovery compounds are conducted in mice to support subsequent pharmacokinetic/pharmacodynamic studies. Typically a single pharmacokinetic profile of a discovery compound is obtained by dosing between 2 to 3 mice and collecting a total of 7 to 9 time points of blood over a 24-h period (3 to 4 time points of blood collected per mouse). This approach is referred to as a nonserial blood sampling technique and results in increased compound and animal use. In addition pharmacokinetic variability is also increased. With the advent and refinement of high sensitivity LC/MS/MS methods for drug level quantitation, opportunity exists to reduce and refine the current paradigm. We report a serial blood sampling methodology in a single mouse that uses various blood sampling routes such as retroorbital, cheek, and saphenous venepuncture. Two compounds (compound A and B) were chosen and subjected to oral and intravenous dosing in mice. Approximately 7 to 8 blood time points were collected by both serial and nonserial blood sampling methods over a 7-h period. Serum samples were analyzed by LC-MS/MS. Pharmacokinetic parameters, such as volume of distribution, clearance, exposure, half life, and bioavailability for each compound, were determined for both mouse blood sampling techniques. Overall, the in vivo PK values obtained after intravenous and oral administration were comparable between both blood sampling (< 2-fold) methods for the 2 compounds tested. The application of the new method has led to a reduction in animal and compound usage, an increase in throughput and speed, and decreased data variability using serial blood sampling techniques.

### P235 Evaluation of the Individually Ventilated Cage Microenvironment for the Laboratory Opossum (*Monodelphis domestica*)

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The laboratory opossum (*Monodelphis domestica*) is the most commonly used marsupial in biomedical research. Its small size, resistance to disease, and birth at an early developmental stage makes the laboratory opossum a valuable model in studies of behavioral evolution, developmental biology, xenograft transplantation, and cholesterol-induced hyperlipoproteinemia. At our institution, laboratory opossums are housed in (35.6 cm × 25.4 cm × 17.8 cm) polycarbonate, individually ventilated cages designed for rats. Previous studies of the cage microenvironment of rodents housed within individually ventilated cages have demonstrated that the cage change frequency could be extended from 7 d to 14 d, without detriment to the animals' wellbeing. We sought to determine if the laboratory opossums housed in individually ventilated cages could have the cage change frequency extended for up to 14 d. Animals were placed into 3 experimental groups: singly housed males, singly housed females, and females housed with litters. The 14 d testing period was repeated twice, with temperature, humidity, and ammonia levels tested on day 0 (cage change), day 7, and day 14 (cage change). Acceptable ranges for the cage microenvironment were based upon standards followed by our institution for housing rodents: temperature between 22 to 26 °C, humidity between 30% to 70%, and ammonia less than 25 ppm. For the duration of both 14 d testing periods, singly housed males and singly housed females had temperature, humidity, and ammonia levels that were within the acceptable ranges. However, females with litters had significantly ( $P < 0.0001$ ) elevated levels of ammonia (greater than 25 ppm) at day 7 for both testing periods and subsequently were changed out of their cages. In summary, the cage change frequency for a singly housed laboratory opossum in an individually ventilated cage can be extended to 14 d while maintaining appropriate cage temperature, humidity, and ammonia levels.

### P236 Diagnosis Method Based on Sandwich ELISA Using *Helicobacter hepaticus*-Specific Monoclonal Antibody

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*Helicobacter hepaticus* infection is prevalent in laboratory mice and causes chronic hepatitis and colitis. Although PCR test is currently the favored diagnostic tool for *Helicobacter* infections in mice, verification procedures to confirm specificity of the PCR products are necessary. We aimed to develop a sandwich ELISA method for detection of *H.hepaticus* antigens by using *H.hepaticus*-specific monoclonal antibodies (MAbs). Mice were immunized with formalin-inactivated *H.hepaticus* and used for preparing MAbs. The reactivity of MAbs was screened by ELISA using inactivated *H.hepaticus*, *H.bilis* and *H.muridarum* antigens. The specificity and sensitivity of the MAbs to detect *H.hepaticus* antigens were further analyzed by sandwich ELISA using anti-*H.hepaticus* rabbit polyclonal antibody as a solid-phased capture reagent and anti-*H.hepaticus* MAbs as a detection reagent for captured antigens. In addition, immunoblot analysis was conducted to determine the molecular masses of the antigens recognized by the MAbs. MAbs specific to *H.hepaticus* as well as those cross-reactive to *H.bilis* and *H.muridarum* were produced. One of the *H.hepaticus*-specific MAbs, clone #5-12D, detected at least 0.00004 OD<sub>600</sub> ( $6 \times 10^4$  CFU/mL) of *H.hepaticus* culture by sandwich ELISA. In immunoblot analysis, the specificity of the MAb was confirmed and the molecular mass of the antigen recognized by MAb #5-12D was estimated to be 12KDa. When mouse fecal pellets spiked with *H.hepaticus* cells were analyzed, sandwich ELISA could detect *H.hepaticus* cells in the fecal samples with the same sensitivity ( $6 \times 10^4$  CFU/mL) as that detectable by PCR. These results suggest that the sandwich ELISA method using MAb #5-12D has potential for specific and sensitive detection of *H.hepaticus* infection in mice.

### P237 Modified Skin Window Technique in Cynomolgus Macaques for Assessing Neutrophil Extravasation

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Alterations in neutrophil extravasation are seen in disease states and in response to therapeutics. Skin window techniques have been used to investigate the acute inflammatory response and changes in neutrophil extravasation in vivo. Our aim was to develop a nonhuman primate model of neutrophil extravasation by adapting a skin window technique used in humans to the cynomolgus macaque. Modulation of neutrophil extravasation was accomplished using an antiinflammatory dose of methotrexate (MTX) to reduce extravasation and local application of the anaphylatoxin C5a to increase extravasation. On day 1, 4 monkeys (under ketamine anesthesia) had skin windows created on both forearms by mildly abrading the skin and overlaying the abrasion with papers either saturated in normal saline or recombinant C5a (rC5a) for 6 h post abrasion. On day 2, the monkeys were given 4.5 mg MTX intramuscularly 2.5 h prior to the abrasions. On day 2, the skin window technique was repeated on new forearm site (day 2 MTX + saline and rC5a) on each monkey. All papers were analyzed for albumin level, neutrophil number, and the neutrophil chemottractant IL-8. The response per paper for day 1 saline was  $2.97 \pm 0.24$  mg of albumin,  $13.58 \pm 0.26 \times 10^6$  neutrophils, and  $7.98 \pm 1.72$  ng of IL-8. No significant differences in albumin levels were observed between any groups, indicating a consistent degree of mild abrasion. MTX given prior to the day 2 abrasions significantly ( $P < 0.05$ ) reduced neutrophil extravasation and IL-8 level compared to day 1 (day 2 MTX + saline  $4.42 \pm 1.71 \times 10^6$  neutrophils/paper,  $2.62 \pm 1.19$  ng IL-8/paper). rC5a abrogated some of the MTX-induced reduction ( $P < 0.05$ ) in the degree of neutrophil extravasation and IL-8 production ( $10.21 \pm 0.11 \times 10^6$  neutrophils/paper,  $6.57 \pm 2.83$  ng IL-8/paper). This technique was well tolerated by the monkeys and successfully demonstrated that this skin window technique was capable of measuring changes in neutrophil extravasation in response to inflammatory modulators.

### **P238 Neural Progenitor-Specific Oncogenic Kras Expression in Cre/Lox-Regulated Transgenic Zebrafish**

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Ras proteins regulate signaling pathways that control many cellular responses such as proliferation, survival, and differentiation. However, there are intriguing questions about the relationship between the developmental timing of specific mutations and the resultant phenotypes in individual cells. In this study, we used the Cre/loxP system for maintaining transgenic zebrafish lines harboring oncogenic KrasV12 under nestin promoter, and investigated the developmental effects of Ras activation in neural progenitor cells. Activated human KrasV12 was induced within pDSNesLCherryLEGFPKrasV12 transgenic fish by Cre mRNA injection. Cre-mediated gene excision was confirmed by PCR and the injected embryos were investigated for the Ras effects using the HE staining, TUNNEL assay, and in situ hybridization. pDSNesLCherryLEGFPKrasV12 transgenic embryos normally expressed mCherry in their central nervous system throughout the developmental stage. However, Cre mRNA injection efficiently excised the flanking stop sequence and the injected embryos expressed EGFP in their brain with severe edema. Brain histology showed that neuronal cell differentiation could occur in spite of oncogenic Ras overexpression, but massive apoptosis and brain edema caused embryonal early death. In summary, overexpression of KrasV12 induced extensive apoptosis of neural progenitor cells followed by severe edema of the brain. But some of the neural progenitor cells resisted to the KrasV12 and could retain their ability to differentiate into neurons. Finally, our transgenic model demonstrated the inability of KrasV12 alone to induce brain tumor at the early stage of development on zebrafish.

### **P239 Potential Toxicity of Glucosamine Mediated through Transforming Growth Factor $\beta$**

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Osteoarthritis, a degenerative joint disease, is the most common form of arthritis affecting at least 20 million Americans, a number that is expected to double over the next 2 decades. While it is estimated that up to 5 million Americans have consumed the dietary supplement glucosamine as a treatment for joint pain or as a prophylactic for healthy joints, its efficacy, safety, and mode of action remain controversial. In this study, 5 concentrations of glucosamine (15, 30, 120, 300, and 600 mg/Kg/d, oral gavage), separate and in combination with chondroitin sulfate, were given to lean Zucker rats for 6 wk. While such treatment did not significantly alter serum insulin and leptin levels, urinary glucosamine concentrations, detected by HPLC, were increased by oral glucosamine treatment. Glucosamine (300 or 600 mg/kg/d) increased transforming growth factor  $\beta$  (TGF $\beta$ ) mRNA content in liver and kidney tissues. Two-fold upregulation of TGF $\beta$  mRNA was detected by real time PCR in liver of the rats sacrificed at 1 and 4 h after the last treatment. A 2-fold increase in TGF $\beta$  mRNA expression in kidney from rats sacrificed at 4 h after the last glucosamine (600 mg/Kg/d) treatment was also detected. TGF $\beta$  is known to stimulate extracellular matrix formation in responsive cell types. While increased extracellular matrix can be beneficial to damaged cartilage in arthritic joints, it can also produce pathogenic sclerotic conditions in kidney.

### **P240 A Preliminary Evaluation of Single Nucleotide Polymorphism Analysis for Routine Genetic Monitoring of Outbred Rats**

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Current methods for monitoring the genetic integrity of strains of laboratory mice (*Mus musculus*) and rats (*Rattus norvegicus*) have focused on ensuring that inbred strains have not become contaminated through mismating. In contrast,

outbred rodent stocks are not commonly monitored using traditional molecular techniques because of their inherent high levels of genetic variation. There is a growing concern regarding the allelic frequencies among colonies of the same stock housed in different locations. This is especially true if an outbred stock has not been maintained as a global model using the same breeding methods worldwide. To determine if a practical approach to monitoring the allelic frequencies in outbred rat subpopulations was feasible, we examined the allelic frequencies among 4 subpopulations of the RccHanTM:WIST outbred rat and 15 subpopulations of the outbred Hsd: Sprague-Dawley rat. While all RccHanTM:WIST rat colonies have been bred globally using a 12 section Poiley rotational mating system, the Hsd: Sprague-Dawley has not historically been maintained as a global model, and in some cases, subpopulations have been separated for more than 10 y. We describe a technical methodology and data analysis approach to evaluate a panel of 96 single nucleotide polymorphism (SNP) markers using Taqman allelic discrimination chemistry on the Fluidigm BioMarkTM system for routine genetic characterization on different subpopulations of these strains ( $n$  = approximately 48). Statistical analysis of heterozygous allelic frequencies was performed using a contingency  $\chi^2$  test and indicates that there was no significant difference ( $P \leq 0.05$ ) found among the number of heterozygous loci among the 4 RccHanTM:WIST subpopulations tested. Conversely, this same statistical analysis revealed that there were significant differences in the level of heterozygosity found among several of the 15 Hsd: Sprague-Dawley subpopulations. These data demonstrate that using SNP analysis for routine genetic monitoring of outbred rat subpopulations can be a powerful tool in evaluating breeding methods, monitoring genetic drift, and comparing allelic frequencies over time in both globally and nonglobally maintained rodent models.

### **P241 Allelic Frequency of the Aryl Hydrocarbon Receptor (AhR) Mutation in the RccHanTM:WIST Rat**

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It has been reported in the literature that the Hannover Wistar rat model traditionally used in toxicology research and offered by several commercial animal vendors, carries a mutation in the aryl hydrocarbon receptor gene (AhR). A point mutation (AhR) in the gene for this receptor results in an altered response to dioxin and other aromatic hydrocarbons. Although the mutation seems to have a limited impact on the overall phenotype of these rat models, the allelic status of this gene could be of importance to investigators studying dioxin sensitivity and 2, 3, 7, and 8-tetrachlorodibenzo-p-dioxin (TCDD) metabolism. To verify the allelic frequency of the AhR mutation in the RccHanTM:WIST rat, samples ( $n$  = approximately 60) from each of 4 RccHanTM:WIST colonies bred in Switzerland, the Netherlands, Japan, and the United States were characterized. Control Crl:WI (Han) samples ( $n$  = 24) from a vendor that had availability of previously published AhR mutation allelic frequency results for this colony, which is reported at 64%, were chosen for comparison studies. Single nucleotide polymorphism (SNP) genotyping was performed using Taqman allelic discrimination chemistry on the ABI 7900 HT sequence detection system. Results demonstrated the allelic frequency of the AhR mutation to be similar among all 4 colonies tested: Switzerland = 76%, the Netherlands = 66%, Japan = 76%, and the United States = 78%. In comparison, the Crl:WI (Han) control samples demonstrated a higher frequency (92%) of the AhR mutation as compared to the RccHanTM:WIST stock. These results demonstrate that the RccHanTM:WIST rat carries a mutation in the aryl hydrocarbon receptor gene in frequencies that are expected for this model, and that there has been no significant genetic drift in the AhR locus among the 4 colonies tested. Although the allelic status of this gene may be important for investigations involving dioxin metabolism, the AhR mutation is considered to be normal for the Hannover Wistar outbred rat. Continued maintenance of this mutation should occur to avoid selection pressure that could result in genetic and phenotypic changes of the RccHanTM:WIST model.

### **P242 Evaluation of a 17.8 kbp Deletion in the Nicotinamide Nucleotide Transhydrogenase (Nnt) Gene in Harlan Laboratories' C57BL/6 Substrains and Related Mutant Models**

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It has been widely reported in the literature that the C57BL/6J mouse substrain carries a deletion in the nicotinamide nucleotide transhydrogenase (Nnt) gene. This mutation is due to a 17.8 kbp deletion in the Nnt gene which involves exons 7 to 11. The absence of the NNT protein has been associated with impaired glucose homeostasis control and reduced insulin secretion and is also required for normal mitochondrial function including metabolism and protection from oxidative stress. To characterize the genetic status of the nicotinamide nucleotide transhydrogenase (Nnt) gene in our C57BL/6 substrains and related mutant models, we performed genetic testing animals from each colony (N=5) using polymerase chain reaction (PCR) analysis. The substrains tested included C57BL/6NHsd, C57BL/6JOLAhsd, C57BL/6JRccHsd, B6.V-Lepob/OlaHsd, and BKS.Cg +Leprdb/+Leprdb/OlaHsd. Two sets of primers were used to confirm the absence or presence of the Nnt mutation in mouse tail biopsy tissues. One set of primers were positioned on the outside of exons 7-11 and the other set was positioned on the inside of exons 7-11 which spanned the breakpoint of the mutation. PCR products were run on a LabChip90 high throughput robotic bioanalyzer and scored for band size. Animals who did not carry the Nnt deletion demonstrated a 312 bp with the first set of primers and no band with the second set of primers. Animals who did carry the Nnt deletion demonstrated no band with the first set of primers and a 547 bp band with the second set of primers. Our results indicate that none of the C57BL/6 substrains or related mutant strains tested carry the 17.8 kbp deletion in the Nnt gene that is found in the C57BL/6J substrain.

#### P243 A Study of Fluid Sterility Once the Seal Is Broken

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A study was conducted to determine if IV fluid sterility is maintained once the bag has been manipulated. Literature searches found little published information on how long IV fluids should be maintained once they have been opened and punctured. The study design included reviewing multiple types of fluids: 0.9% sodium chloride, sodium chloride with 5% dextrose, and lactated ringers. These were tested under different environmental storage conditions: room temperature (20 to 22 °C), incubator (39 °C), and refrigeration (2 to 5 °C). A standard amount of fluid was allowed to pass through the tubing to simulate routine subcutaneous or intravenous administration use. Each day fluid was removed from the bags via the injection port with a needle and syringe using aseptic techniques. Weekly the fluid was allowed to flow through the line and was collected for bacterial culture using blood agar plates. A palm sized luminometer unit was used to monitor bacterial concentrations via ATP detection. A positive control was compared using a *Staphylococcus aureus* spiked bag under identical conditions and was assessed using the same methods post inoculation. The results show that a common bacterial contaminate does not seem to compromise the sterility of these fluids and the colony count actually decreased during time under all 3 housing conditions. Beginning on day 7, the bacterial count in the sodium chloride bags with and without dextrose, and lactated ringers warm and room temperature had a significant decrease in their bacterial counts. The lactated ringer stored under refrigeration had a noticeable increase in the bacteria count. At days 14, 21, and 28 results show that the bacteria count decreased significantly in all solutions. The fluid bags that did not receive the experimental inoculums showed no growth on the blood agar culture plates, and the testing unit results were negative through 45 d. This experiment demonstrates that fluids maintain sterility even after routine manipulation. This is possibly due to lack of air, or the preservatives added for this purpose. Due to the current economy and the reduction of funding for the research community, these findings could help decrease the waste of changing these fluids on a regular basis.

#### P244 A Novel Mouse Model for Agenesis of Corpus Callosum

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Agenesis of the corpus callosum (ACC) is an abnormality of brain structure present at birth that is characterized by partial or complete absence of the corpus callosum. It occurs in 1:4000 humans. Genetically induced mutant mice provide us critical insights of developmental and molecular basis of

ACC. We previously developed a mutant mouse line bearing a transgene insertional mutation. We named it TAS mouse line. TAS mice with a genetic background on BALB/cAJcl were carried Tet-on expression system-relative genes. In the current study, we characterized TAS mice with agenesis of the corpus callosum. On the morphologic investigation of brain structure, TAS mice showed partial absence of corpus callosum accompanied with formation of Probst Bundles leading abnormality of cerebroventricular formation. Sixty-six percent of the heterozygous adult mutants (19 of 29) developed ACC. In contrast with TAS mice, the abnormality was absent in 53 nontransgenic mice with a genetic background on BALB/cAJcl. There is no report that Tet-on expression systems affect mouse development. It seems that the cerebral abnormalities are due to transgene insertional mutation. To detect the transgene insertion site, we performed multiple FISH analyses. On the 1 color FISH analysis, we detected the transgene signals on chromosome 18. On the 2 color FISH analysis, transgene signal was observed in a block from 12.9 to 16.9 Mb on chromosome 18. We were successful in narrowing the chromosomal region (12.9 to 16.9 Mb on chromosome 18) containing the transgenes. There might be a novel gene associated with ACC in the candidate chromosomal region. Future study will be required to investigate the cerebral expression of candidate genes for ACC on TAS mice.

#### P245 An Experimental Mouse Model of Human Necrotizing Enterocolitis Roles of Toll-Like Receptor 4 and 9 in Pathogenesis and Associated Treatment

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Necrotizing enterocolitis (NEC) is the most common gastrointestinal disease of premature infants and a major cause of morbidity and mortality in neonatal intensive care units. Risk factors for its etiology include prematurity, hypoxia, enteral feeding, and bacterial colonization due to unhygienic conditions. We developed a mouse model mimicking the clinical disease to study the pathogenesis and treatment. Experimental NEC was induced in 10-d-old mice, which were gavaged fed (advanced infant formula: canine milk replacer at a ratio of 2:1) 5 times daily and exposed to intermittent hypoxia (5% O<sub>2</sub>, 95% N<sub>2</sub>) for 10 min using a modular hypoxic chamber twice daily for 4 d. Animals were fed 200 µL per 5 g of mouse body weight by gavage over 2 to 3 min using aseptic 24-French angiocatheter that was placed into the mouse esophagus under direct vision. Non-NEC control animals remained with their mothers and were fed breast milk. Experimental mice were gavaged with recombinant adenoviruses expressing dominant negative TLR4 twice daily for 3 d (240 µL; 1012 PFU), and CpG-DNA (1 mg/kg daily for 4 d) as therapeutic agents. We observed an aberrant increase in TLR4 mRNA expression (2.5-fold versus control, *P* < 0.05) and protein levels (2.8-fold versus control, *P* < 0.05), and decrease in TLR9 mRNA expression (4-fold versus control, *P* < 0.05) in our mouse model. A similar increase was observed in TLR4 mRNA (3.3-fold versus control) and protein levels (2.9-fold versus control) in human NEC tissues. Also, we have observed a decrease in the severity of experimental NEC afforded by dominant negative TLR4 adenovirus (measured as NEC pathologic score: control 0.2 ± 0.01, NEC 2.4 ± 0.5, dominant negative TLR4 administered mice induced for NEC 0.8 ± 0.2). Oral administration of mice with TLR9 modulator CpG-DNA (1 mg/kg) reduced the incidence of NEC from 30% to 10%. Findings demonstrate that our experimental protocol induces intestinal inflammation and the release of proinflammatory cytokines in a pattern that closely resembles human NEC. An aberrant increased signaling of TLR4 appears to be responsible for the pathogenesis of NEC, and treatment with drugs such as CpG may be useful for the prevention and treatment of human NEC.

#### P246 Adrenal Weight as a Measure of Chronic Stress in Chickens

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Optimization of housing conditions for laboratory animal species typically focuses on a combination of husbandry, practicality, and minimization of animal stress. While metrics for husbandry and practicality are readily quantifiable, quantification of stress parameters, such as adrenal hormones and behavioral testing, can be readily influenced by animal handling, social interaction, and the sampling paradigm. In avian species, feather loss is



known to result from self or conspecific trauma. Regardless of the source, avian feather loss is a quantifiable indicator of environmental stress. In chickens, feather loss severity is related to aggressive conspecific pecking and large groups of birds. Given that adrenal remodeling has been found to be correlated to chronic stress in select species, we hypothesized that adrenal remodeling would be proportional to feather loss in chickens. Specific pathogen-free (SPF) chickens (P2A strain) were group housed in continuous breeding colonies (20 birds per colony at 1.2 ft<sup>2</sup>/bird; rooster:hen ratio = 1:9). Seventeen adult hens (77 wk old) from this housing paradigm were evaluated for correlation between feather loss and right adrenal gland weight. We found that adrenal weight (AW) and the ratio of AW to body weight (A:BW) were both strongly correlated to total feather loss scores (TFLS;  $r = 0.77$  and  $0.84$ , respectively). However, neither TFLS nor AW were correlated to body weight ( $r = 0.14$  and  $0.42$ , respectively). Histopathology of adrenal glands from the lower and upper 25th weight percentiles (0.88 and 0.153 g, respectively) illustrated medullary and cortical hyperplasia with a relative preferential increase in cortical cell populations (cortical:medullary ratio = 0.33 and 0.47, small and large gland, respectively). These findings illustrate adrenal hyperplastic remodeling that is proportional to feather loss in chickens. The concurrence of these 2 parameters indicates the adrenal remodeling observed resulted from chronic stress. Thus, adrenal weight is a quantitative parameter for assessment of chronic stress in chickens. This finding has potential for application to other lab animal species and will serve as an objective parameter for optimizing welfare in our chickens.

#### **P247 Intermediate Noise Intensity Results in Growth Abnormalities in Chickens**

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Exposure to environmental noise is known to cause a wide range of abnormalities in laboratory animal species as well as human beings. In spite of the extensive reports on noise-induced pathology in mammalian species, there is a paucity of data regarding auditory-induced pathology in avian species. Anecdotal observations with a novel poultry dander collection system suggested the system was associated with reduced weight gains and possibly increased mortality rates in hatchling chickens. A prospective study was conducted to evaluate both effectiveness and impact of the dander collection system. Three groups of leghorn (P2A strain) chicken hatchlings ( $n = 29$  per group) were raised from 1 to 42 d of age in each of 3 different HVAC paradigms, HVAC system A (A; 100% fresh air, 12 ACH, constant ambient noise level = 54 dB), system B (B; 100% fresh air, 16 ACH, constant ambient noise level = 64 dB), and system B with a dander collection device (Bd; 100% fresh air, 22 ACH, constant noise level = 72 dB). Animal census was tabulated daily while body weight was collected at weeks 2, 4, and 6. Air particulate levels were measured at times 0, 2, 4, and 6 wk (T = 0, 2W, 4W, and 6W, respectively). At week 6, all birds were humanely euthanized and visceral weights were obtained. While the dander collection system was effective, it was associated with a higher mortality rate (all cause mortality survival rates = 52%, 97%, and 100% for groups Bd, B, and A, respectively). Mean body weight for group Bd differed significantly from groups A and B at all time points (2W BW = 61, 74, and 80, respectively; 4W BW = 119, 159, and 167, respectively; 6W BW = 219, 304, and 303, respectively;  $P$  value for Bd versus A and B at 2W, 4W, and 6W:  $P < 0.001$ ,  $P = 0.001$ , and  $P < 0.05$ , respectively). Left ventricular and splenic weights were significantly lower in the Bd and B groups. In summary, significant mortality and growth retardation occurred in the growing chickens raised with an ambient noise level of 72 dB. The impact of elevated noise was overwhelming relative to any benefit that may have resulted from reducing the air particulate levels. These findings indicate that OSHA accepted noise limits ( $\leq 85$  dB) are not appropriate for all nonhuman species.

#### **P248 Investigating the Anxiolytic Effects of Pregabalin in Unanesthetized Rats**

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Pregabalin (PGB) is a ligand for the alpha-2-delta subunit of voltage-gated calcium channels and is currently prescribed for pain from fibromyalgia and diabetic neuropathy. Recent studies have shown that PGB also exhibits

anxiolytic effects. Previously we have shown that stress, such as that from a tail pinch, elevates the levels of extracellular glutamate in the rat prefrontal cortex (PFC). Here we examine the effects of PGB on stress-evoked glutamate release in the PFC of unanesthetized rats, hypothesizing that stress-evoked glutamate levels will be lower in animals pretreated with PGB than in animals pretreated with saline (Veh). Microelectrode arrays (MEA) were assembled and prepared for in vivo amperometric recordings as previously described. Male, 12-wk-old F344 rats were anesthetized and implanted with the MEA pedestal assembly into the PFC and monitored after surgery. Glutamate levels were recorded on 3 consecutive days starting on day 3 after implantation. On each day of recording, rats received no treatment, Veh, or 30 mg/kg PGB 1 h before receiving a 5-min tail pinch stressor. Total stress-evoked glutamate release during the 5-min tail pinch was recorded and analyzed using a 2-tailed  $t$  test. Glutamate release after pretreatment with PGB was  $91 \pm 8\%$  of resting levels. Glutamate levels after Veh pretreatment and stress ( $122 \pm 10\%$  of resting levels) were significantly higher than in animals pretreated with PGB ( $P < 0.05$ ,  $n = 6$ ). These findings support our hypothesis that glutamate release in response to tail pinch stress is attenuated in the presence of PGB and, therefore, suggest that the compound may be effective in alleviating stress-related anxiety.

#### **P249 Transdiaphragm Surgical Approach for Implantation of Pulmonary Artery Pressure Telemetry in a Pulmonary Hypertension Rat Model**

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Pulmonary hypertension is characterized by elevated pressure in the pulmonary arteries. To better understand its physiopathology, and to test potentially useful therapeutic compounds, several rodent models, including the telemetry implantation model, have been developed. The major challenge with these models is that they either lack a reliable methodology to continuously measure pulmonary arterial pressure (PAP) and/or require major invasive thoracotomy and laparotomy surgical approaches, which may impair the cardiovascular system. Therefore, the goal of this study was to develop a method allowing continuous PAP monitoring in conscious, freely moving rats utilizing a less invasive, repeatable surgical procedure. For this purpose, we used a live feed telemetry trace during catheter placement to accurately position the telemetry probe in the pulmonary artery. An abdominal incision was made to allow for a transdiaphragmatic surgical approach to implant the PAC-10 transmitter catheter through the right ventricle into the pulmonary artery. With the presence of the live feed telemetry trace, we were able to repeatedly place the catheter tip into the pulmonary artery, achieving success rates greater than 80%. Two weeks after postsurgical recovery, the mean pulmonary arterial pressure (mPAP) was successfully and reliably measured in conscious, unrestrained rats. The mPAP and pulse pulmonary arterial pressure (pPAP) were sustained at 21.7 to 20.2  $\pm$  1.5 mm Hg and 18.0 to 19.0  $\pm$  1.4 mm Hg, respectively. Three months after the surgery, necropsy and histopathology of 6 rats demonstrated that the PAC-10 telemetry transmitter placement was reliable and did not lead to histopathologic damage of the endocardium of the right ventricle and pulmonary valve. The transdiaphragm surgical approach was determined to be easier, repeatable, and less invasive than the more common thoracotomy and laparotomy method.

#### **P250 A Mouse Model for Human Atopic Dermatitis Using DNCB Patch**

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Atopic dermatitis (AD) is the chronic and recurrent inflammatory skin disorder by genetic, environmental, and microbial factors. Among murine models of human AD, the model of repeated hapten (TNCB, DNCB, or oxazolone) applications has many benefits due to being reproducible and inexpensive. However, the previous hapten-induced model has some limitations because applying the same size hapten on each mouse is difficult and the mouse could easily eat it during grooming. Therefore, we tried to overcome them and establish an AD murine model by repeating the application of DNCB patch in BALB/c mice, which has advantages in reproducibility and low expense. For inducing AD, the backs of BALB/c were shaved with electric clipper and depilatory cream, and washed with sterilized PBS-gauze 1 d before sensitization. For the sensitization, a 1 cm<sup>2</sup> gauze-attached patch, where 100  $\mu$ L 1% DNCB in acetone-olive oil (AOO) or AOO applied alone, was attached on the shaved

back for 48 h on day 0 and 3. On day 7 and 10, 100  $\mu$ L 0.2% DNCB in AOO or AOO alone as the sensitization method had been challenged for 24 h. To estimate how homologous our animal model with human atopic dermatitis compared to the previous popular AD models, NC/Nga mice, we evaluated clinical, histologic, and immunologic alterations. The model showed an increase of erythema, excoriation, subiliac lymph node weight, mast cells, epidermal hyperplasia, and serum IgE levels. In the expression of mRNA, the IL-4/IFN- $\gamma$  ratio appeared high. These data demonstrate our DNCB-patch model represents human AD-like lesions, not only the skin erythematous plaques, eruption, epidermal hyperplasia, and the increased infiltration of mast cell, but also the hyperproduction of IgE. We anticipate that our model might be used in studying the etiology, pathology, and treatment of human AD.

#### **P251 Role of $\beta$ -Catenin in N-Methyl-N-Nitrosurea-Induced Mouse Gastric Cancer**

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To assess the role of  $\beta$ -catenin in stomach cancer, we performed pathologic and immunohistochemical analysis in C57BL/6J mice given drinking water containing 200 ppm of N-methyl-N-nitrosurea (MNU) on alternate weeks for 10 wk. The mice were euthanized 48 wk after first administration. In

histopathologic analysis, the incidences of gastric adenomas and adenocarcinomas in MNU-treated mice were 60.5% (26 of 43) and 27.9% (12 of 43), respectively. But neoplastic lesions were not observed in untreated mouse (0 of 20). In normal stomach, cyclin D1 was not stained, but  $\beta$ -catenin was partially expressed in membrane of epithelial cells associated E-cadherin. Nuclear expression of  $\beta$ -catenin was observed in 46.2% (12 of 26) of adenomas and in 91.7% (11 of 12) of adenocarcinomas. Similarly, 38.5% (10 of 26) of adenomas and 83.3% (10 of 12) of adenocarcinomas exhibited nuclear localization of cyclin D1 in the same area where  $\beta$ -catenin expression occurred. Osteopontin was partially expressed in normal gastric chief cells and mucous cells. But, normal expression of osteopontin disappeared in proliferative lesions, and expression of osteopontin in gastric tumors was observed in 84.6% (22 of 26) of adenomas and 100% (12 of 12) of adenocarcinomas. Intensity of osteopontin expression was increased in accordance with cancer progression. In this study, the expressions of  $\beta$ -catenin, cyclin D1, and osteopontin were observed in the same tumor and their expressions were associated with gastric adenocarcinoma rather than adenoma. Integrating with expression ratios and patterns, although not all of cyclin D1 and osteopontin expression were associated with  $\beta$ -catenin,  $\beta$ -catenin overexpression through activated Wnt pathway or loss of E-cadherin may induce cyclin D1 and osteopontin, and play a important role in MNU-induced mouse gastric cancer, especially in advanced cancer.