

# Abstracts of Scientific Papers

## 2018 Association of Primate Veterinarians Workshop

### The Non-chair Device For Primate Restraint

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Ask any primate veterinarian about the chair restraint of macaques for procedures and you will hear about the numerous constraints that they need to overcome before an animal can sit calmly (if ever) in that device. Several refinement attempts have been made to better this restraint device and a recent survey indicated that 2/3 of laboratories use a closed box-chair and the remaining 1/3 train to go in the open-chair design. In our culture of continuous animal welfare refinement, the team devised a multifunctional detachable restraint chamber for procedures with macaques in the research environment. This detachable module can be fitted to any existing macaque cage design and requires very little training for the monkey to sit in. The arms and legs of the animal are not restrained in this module (cause of instinctive struggling in animals) and the different windows allow access to various key areas of research interest. The multifunctionality of the device allows it to be used for various other routine procedures like weighing, blood draw, trans abdominal palpations, and even regular daily treatment for hospitalized animals.

### Oxytocin and Pair Compatibility in Rhesus Macaques

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Pair housing is considered one of the best ways of promoting psychological wellbeing for caged macaques. However, if the partners are not compatible, it can result in stress or aggression. While studies have analyzed the role of variables such as weight, age, and gender on pair success, few have examined the relationship between physiological parameters and pair compatibility. Oxytocin is known to promote affiliative nonsexual behavior in various primate species and may serve as a potential indicator of pair compatibility. The goal for this study was to examine the correlation between oxytocin and prosocial behaviors in isosexual male pairs of rhesus macaques. We hypothesized that there would be a positive correlation between oxytocin and social behavior. We collected blood oxytocin samples on 28 pairs of monkeys (n=56) that had lived together for at least 1 mo. We simultaneously collected behavioral data on the pair using focal sampling techniques (8, 10 min observations). Behaviors coded included close social contact, grooming, proximity, and aggression. Oxytocin varied among individuals, but was highly correlated between members of a pair ( $r = 0.85$ ,  $P = 0.0$ ). Additionally, monkeys in pairs that demonstrated high prosociality had significantly higher oxytocin than from pairs with low levels of social behavior ( $F(1,54) = 6.4$ ,  $P = 0.015$ ). We are currently examining this relationship in females. Our results

suggest that oxytocin may play a role in the quality of isosexual pairs of male macaques.

### Sterile Abscess Formation and Delayed Wound Healing in a Rhesus Macaque (*Macaca mulatta*)

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Use of sustained release (SR) opioid and NSAID analgesics have demonstrated efficacy allowing prolonged relief from pain between dosing when compared with standard formulations. By removing the need for multiple injections, these SR formulations minimize animal handling related stress and tissue trauma, and increase safety for the handler. Unfortunately, adverse effects such as injection site reactions, have been reported following administration of buprenorphine SR and meloxicam SR in various species. A 9-y-old, 10 kg, male rhesus macaque (*Macaca mulatta*) with protracted healing of a head cap skin margin, received subcutaneous injections of buprenorphine SR on 5 separate occasions over a period of several weeks. The doses were administered in different locations, after corrective procedures. Four sterile abscesses formed, as evidenced by culture and histopathology, each in close proximity to the injection sites. All but 1 abscess resolved with proper cleaning and drainage. The remaining abscess developed into an open wound, which was initially managed by resection of devitalized tissue. The resected area remained unresponsive to conservative treatment consisting of sensitivity directed systemic antibiotics, topical antiseptic flushing, and topical antibiotic application, and over a prolonged period required multiple corrective interventions due to dehiscence. Approximately 3 mo after initial resection a new treatment plan was initiated involving second intention healing using topical antibiotics, silver sulfadiazine cream, manuka honey bandages, and the use of a jacket to prevent manipulation of the wound. Over the next 3 mo using the new regimen, the wound slowly decreased in size, ultimately healing entirely. This clinical presentation is suggestive of an injection site reaction in response to administration of buprenorphine SR. Potential etiologies of delayed wound healing have been and continue to be explored.

### Use of a Novel Scoring System to Evaluate a Polyunsaturated Fatty Acid Product for Treating Atopic Dermatitis in Rhesus Macaques

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Atopic dermatitis is a chronic condition that is notoriously difficult to evaluate and treat in various animal species. Although glucocorticoids and calcineurin inhibitors have been efficacious in many of these cases, their use in laboratory macaques can

present a myriad of challenges. Recently, a topical, biodiffusible, polyunsaturated fatty acid (PUFA) product has shown promise in ameliorating the clinical signs of atopic dermatitis in dogs and cats. In this study, 3 rhesus macaques affected with chronic dermatitis, along with age and sex-matched, unaffected controls, were assessed using a novel macaque atopic dermatitis extent and severity index (MADESI); this scoring system was adapted from a similar scoring index widely used in dogs. Full-thickness skin biopsies, photographs, and routine bloodwork were also obtained prior to treatment. A spot-on product was then applied cageside to the dorsum of all animals at weekly intervals for 12 wk, followed by a repeat of the MADESI scoring, biopsies, photographs, and bloodwork. Thereafter, treatments were continued at monthly intervals with MADESI scoring every 3 mo. Prior to treatment, high MADESI scores in the affected macaques mirrored the histopathological abnormalities of their skin biopsies. Following the initial 3 mo of treatment, the atopic animals exhibited a marked reduction in clinical lesion severity, as evidenced by lower MADESI scores, along with decreased acanthosis, hyperkeratosis, and inflammation. In contrast, control animals that underwent treatment had minimal changes in their MADESI scores or histopathology over time. Animals and their cage mates demonstrated no adverse response to the treatments. These results indicate that the MADESI scoring system is a valuable tool for tracking chronic dermatitis in macaque colonies. Additionally, a spot-on PUFA treatment reduced chronic dermatitis and likely improved the quality of life in affected macaques, causing minimal stress to the animals or effect on research paradigms.

### Collaborative Generation of Transgenic Marmosets for Neuroscience Research

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While transgenic mouse models of human disease have contributed significantly to many fields of biomedical research, there are important limitations in the ability to model neurologic and psychiatric diseases in the mouse due to differences in brain anatomy and gene expression. Common marmosets (*Callithrix jacchus*) are a small New World nonhuman primate species with high fecundity and early sexual maturation. These characteristics of high reproductive efficiency, along with a close phylogenetic relationship to humans, make the common marmoset ideal for development of NHP transgenic models of human psychiatric illness. Our institution has worked collaboratively with other neuroscience researchers to refine development of transgenic marmoset models. To date, 20 embryo transfers have been performed with a total of 5 pregnancies (pregnancy rate: 25%). Of these 5 pregnancies, 2 live genetically modified offspring have been born. This presentation will discuss cutting-edge assisted reproductive techniques and technologies used successfully in the common marmoset, and will summarize project milestones, technical challenges, and ethical considerations related to the use of transgenic NHP in research.

### Atresia Ani in a Common Marmoset (*Callithrix jacchus*)

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In November of 2015 a juvenile male common marmoset (*Callithrix jacchus*) in good body condition was found dead in his family cage with no previous premonitory signs. Necropsy revealed a gas-distended abdominal cavity, feces-distended large bowel, and a full thickness distal colonic perforation resulting in fecal peritonitis. The distal colon was found to end in a blind pouch 7 mL prior to the expected anal opening (aka atresia ani with imperforate anus). Remarkably, this animal had survived to 83 d-of-age without an apparent way to void digesta. Here we present this case, briefly discuss the origin and correction of such anorectal malformations in the human and veterinary literature, and highlight decisions in husbandry practices needed to identify such devastating congenital defects while limiting disruption and handling of seemingly healthy, young nonhuman primates raised in a complex social setting.

### Testicular Congestion in an Olive Baboon (*Papio anubis*): An Unusual Case Progression

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An approximately 24-y-old, male olive baboon (*Papio anubis*) presented for acute onset of depression, anorexia, and lethargy. Pertinent history includes an indwelling jugular catheter placed in December 2016 and superficial pyoderma (*Staph. aureus*) at the catheter exit-site, which resolved with surgical revision and antibiotic treatment in March 2017. At presentation, the catheter was patent with no evidence of infection or inflammation. Upon physical exam, the right scrotum was found to be enlarged. Ultrasound revealed an enlarged right testicle and spermatic cord with surrounding hyperechoic material and focal, round, hypoechoic areas. Scrotal herniation of omentum or intestines was suspected, and a right sided scrotal exploration revealed an omental herniation and testicular congestion. Hemicastration was performed, the testicle was submitted for histopathology, and supportive care was initiated. Bloodwork abnormalities included mild anemia, thrombocytopenia, a neutrophilic leukocytosis with a left shift, and a moderate hypoalbuminemia attributed to local inflammation, and normal coagulation parameters. Histopathology of the testicle revealed severe suppurative vasculitis with numerous intracellular gram negative rod bacteria, confirming a diagnosis of sepsis with secondary testicular congestion. Treatment with intravenous broad spectrum antibiotics was prescribed. Repeat bloodwork demonstrated progressive changes including a moderate regenerative anemia, moderate thrombocytopenia, and severe neutrophilic leukocytosis with a left shift. Coagulation panel revealed elevated d-dimers and fibrinogen, raising concern for DIC. Although blood cultures were obtained, no growth as found. Due to the grave prognosis and the onset of dependent edema, euthanasia was elected. Necropsy revealed severe mitral valve endocarditis with intralésional bacteria and disseminated suppurative inflammation to multiple organs including the heart, liver, kidneys, spinal cord, and brain. Cultures were obtained of the brain and scrotal sac fluid plus prostate, yielding *Staphylococcus epidermidis* and *Candida albicans*, respectively. Cause of death was determined to be endocarditis with secondary septic thrombosis. Bacteria introduction was attributed to the chronic indwelling catheter, with the presentation of gram-negative orchitis as a secondary process.

## Monocrystalline Iron Oxide Nanoparticle [MION] Sensitivity in a Rhesus Macaque (*Macaca mulatta*)

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Monocrystalline iron oxide nanoparticle (MION) is an exogenous iron-based contrast agent, used for labeling and studying blood and cellular activity in both humans and animals. MION has been utilized to evaluate T cell activity in nonhuman primate (NHP) AIDs models, neural stem cells, tumor cells and macrophage activity, and enhancement of the blood oxygen level dependent (BOLD) signal in fMRI studies of NHPs and rodents. MION injections prior to fMRI imaging, enhance sensitivity between 3 and 10 fold, enabling researchers to make profound strides in understanding the human visual system, motion sensitivity regions and cortical hierarchy of the brain. Using established MION doses ranging from 8 mg/kg up to 60 mg/kg at weekly or greater intervals, has been well tolerated with minimal insult to physiology, behavior, or cognition in NHPs. A 9-y-old, 4.5 kg female Rhesus macaque (*Macaca mulatta*) pair housed with a stable cohort, was enrolled in an IACUC approved study investigating methods to construct a reproducible, functional map of brain activity. Over a course of 200 d the animal experienced 18 awake/anesthetized fMRI exposures with MION doses given IV at 10mg/kg each exposure. In compliance with the protocol, periodic blood monitoring, physiologic and health evaluations were completed, with the possibility of iron chelation. The mean number of days between exposures were 11.5. At the conclusion of this 200 d trial, the animal was observed as having alopecia, and hair picking behavior. Iron panels yielded a remarkably high range of ferritin from 767 ng/dL to 1,328 ng/dL with an average of 907 ng/dL, taken over the 2 mo following study completion. Abnormal blood chemistry and CBC profiles resulted, despite receiving no additional MION, and may be consistent with iron toxicosis or hypersensitivity. This case was unique to the other study animals, as ferritin levels never rose above 421 ng/dL with an average of 222 ng/dL. By the 10th week post study completion, ferritin normalized to 391 ng/dL. Alternative contrast agents, chelation protocols, analysis of blood chemistry, and CBC values will be discussed.

## Guaiac Fecal Occult Blood Testing for Detection of Gastrointestinal Bleeding in the Rhesus Macaque

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Fecal occult blood testing (FOBT) is performed in both human and veterinary medicine to detect gastrointestinal (GI) bleeding. The guaiac-type fecal occult blood test (gFOBT), a qualitative test that detects the peroxidase activity of heme, is commonly used due to low cost and ease of use; nonhuman primate clinicians rely on this test to support the diagnosis of gastric ulcers and GI adenocarcinoma in macaques. Though this test has been assessed in humans, its reliability has not been validated in any nonhuman primate. In this study, we evaluated the ability of the gFOBT to detect bleeding from different regions of the GI tract in macaques. Based on human literature, we hypothesized that this test would be more sensitive in detecting lower versus upper GI bleeding. After

confirmation of a negative prestudy gFOBT, we administered autologous blood into the stomach (5, 10 and 20mL), duodenum (5mL), and colon (0.5mL) of healthy, adult rhesus macaques and analyzed fecal samples using the gFOBT at 24, 48, and 72 h postadministration. The gFOBT detected bleeding from all 3 regions of the GI tract. The sensitivity of the test to detect gastric bleeding was highest at 20 mL (78%) and lowest at 5 mL (44%). Sensitivity to detect 5 mL duodenal bleeding was 100% at all time points. Lastly, the gFOBT detected 0.5 mL of rectal bleeding with 100% sensitivity at 24 and 48 h time points, and overall sensitivity was 78%. These results indicate that the gFOBT is better at detecting lower versus upper GI bleeding in macaques and that it is better at detecting upper GI bleeding than is reported in humans. Still, the amount of blood necessary in the stomach to reach 50% sensitivity may make the test clinically irrelevant for detecting gastric ulcers. Based on these results, we recommend that a negative gFOBT result not be used to rule out upper GI bleeding.

## Refining Treatment of Neonatal Campylobacter Gastroenteritis

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In captive colonies of nonhuman primates, enteric pathogens such as campylobacter jejuni are a common causes of diarrhea. In both humans and macaques, neonates and juveniles are more susceptible to bacterial gastroenteritis and thus more likely to present with clinical signs than immunocompetent adults. This higher morbidity rate poses a unique challenge in a harem breeding colony, especially for still-nursing infants and juveniles. In order to treat infants directly, typically either the mother-infant pair must be separated from the group or the infant separated from the mother. Generally, young lower-ranking monkeys must be removed from the group and separated from the dam in order to treat medically. For juveniles, this risks permanent alteration of group dominance hierarchy and increases the likelihood of fighting with subsequent reintroductions. Even more concerning is the treatment of nursing infants, which either requires repeated sedation of the dam for temporary separation, which increases the likelihood of maternal rejection, or more long-term separation, which risks serious long-term behavioral consequences for the infant. Pharmacokinetic studies in humans have shown that azithromycin is transferred into the breastmilk of lactating mothers at a high rate in a bioavailable form. This offers antibiotic benefit to both the mother and potentially the infant with few reported adverse effects. At our facility, we identified 4 rhesus macaques (*Macaca mulatta*) less than 12 wk of age with clinical signs consistent with campylobacter infection (i.e. failure to thrive and diarrhea). Of these 4 infants, 3 tested positive for campylobacter jejuni via RT-PCR. In all cases the lactating mother was prescribed a course of oral azithromycin for 5 d (250 mg once and 125 mg for 4 consecutive days). Following treatment, all 4 infants were PCR negative for campylobacter jejuni. Clinical signs of all infants resolved following treatment of the dam. Here we present an alternative option to separation of dam and infant for campylobacter treatment. This refinement allows the veterinarian to provide standard of care while maintaining social coherence in the primate colony.

### **Analysis and Comparison of the Intestinal Microbiome in Cynomolgus Macaques (*Macaca fascicularis*)**

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Human gut microbiome research is currently an area receiving much research focus. Characterizing the human gut microbiome associated with particular disease states and diets and the changes that occur in the gut flora has led to better understanding of the human gut microbiome influence on overall human health. Nonhuman primates used in research are known to be susceptible to episodes of diarrhea and inflammatory bowel syndromes similar to those seen in humans. Nonhuman primates with diarrhea may ultimately become unsuitable for use in research resulting in significant impacts financially, scientifically, and on animal welfare and client satisfaction. In order to understand what information can be obtained by analyzing the NHP intestinal microbiome, fecal samples from 2 different origins of NHP (Mauritius and Chinese) were collected and sequenced both at the U.S. supplier prior to shipment, and after acclimation at our facility, prior to study. This database of microbiome information compared males and females, pre- and post-shipment, and the different origins of animals. The type as well as quantity of organisms was analyzed at the different time points for each group of animals. Significant differences in the microbiome composition were found between sexes and in pre- and post-shipment individuals. This type of analysis has the potential to lead to a better understanding of how the intestinal microbiome can change and impact animal health. Identifying changes in the intestinal microbiome could help to intervene through husbandry, veterinary care, enrichment and overall animal care and ultimately help maintain the microbiome in a normal state and result in a healthier nonhuman primate population, and a better research model.

### **The Impact of Dietary Intolerance on the Microbiome in a Small Population of Rhesus Macaques (*Macaca mulatta*)**

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Diarrhea is the most common gastrointestinal disease documented in captive rhesus macaques. The exact pathophysiology underlying chronic diarrhea in nonhuman primates is not well understood, but research supports persistent inflammation. In human medicine, growing clinical evidence indicates the microbiome modulates the severity of inflammatory diseases like irritable bowel syndrome. Furthermore, recent research in humans has shown that restoring the indigenous microbial diversity may be useful in resolving cases of chronic diarrhea when other treatment modalities have failed. The microbiome likely plays a role in the chronic diarrhea exhibited by numerous macaques, and determining differences in bacterial populations may help refine treatment interventions. This study compared the microbiome profiles of 4 clinically normal rhesus macaques to a female rhesus macaque with chronic diarrhea and dietary intolerance. Food enrichment, experimental manipulations, and medical records were maintained at least 1 mo prior to sample collection. Fecal samples were collected for DNA extraction and 16S rRNA sequencing of the V4 region. Bacterial 16S rRNA gene sequences were processed using the software program mothur. Microbial community analysis was

performed using an operational taxonomic unit (OTU) approach where 356 OTUs were characterized between the 5 samples. Firmicutes comprised the vast majority of the phyla in each sample. Other phyla represented were *Proteobacteria*, *Chlostridiales*, *Spirochaetes*, *Actinobacteria*, *Verrucomicrobia*, *Synergistetes*, and *Fibrobacteres*. Alpha diversity, as determined by rarefaction, was lower in the primate with chronic gastrointestinal issues but not statistically significant. Our results suggest microbiome structure may influence susceptibility to chronic diarrhea but additional data is needed. Based on microbiome profile results and possible influencing factors, a survey questionnaire was designed with information on husbandry conditions and health history. Future directions include collaboration with institutions housing large numbers of primates to determine gut microbiota differences. Our results will have an impact on nonhuman primate medicine and animal welfare.

### **Outbreak of Human Metapneumovirus in an Indoor and Outdoor Colony of Rhesus Macaques (*Macaca mulatta*)**

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Human metapneumovirus (HMPV) is a large enveloped single-stranded RNA virus from the family *Pneumoviridae*, previously a subfamily of the *Paramyxoviridae*. HMPV is a respiratory virus with a worldwide distribution and seasonal variation. Usually HMPV affects the upper respiratory tract with clinical signs similar to the common cold: rhinorrhea, sneezing, cough, sore throat, headache, and fever; however in children, elderly and immunosuppressed people, HMPV has been associated with lower respiratory tract disease: bronchiolitis and pneumonia. Here we describe an outbreak of HMPV with secondary bacterial pneumonia in our outdoor and indoor colony of rhesus macaques. This presentation will focus on clinical signs, diagnostic procedures, histopathology, treatment, and containment measures that were taken to contain this outbreak of HMPV.

### **Fetal and Placental Pathologies in Infectious Disease Research**

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The macaque is a well-established model for many infectious diseases and maternal-fetal research has expanded exponentially due to recent outbreaks of zika, listeria, and other pathogens. Medical monitoring during pregnancy often identifies serious and fatal fetal and placental abnormalities caused by infectious diseases but characterization of subtle pathologies requires full post mortem evaluation of the fetus, fetoplacental tissues, and for some investigations, the dam. Congenital zika syndrome is has many adverse outcomes ranging from microcephaly, otic, and ocular defects to pregnancy loss. As with other infectious diseases such as listeria, and *Porphyromonas gingivalis*, the pathogen may have little clinical effect on the dam with variable fetal pathology depending upon the gestational age at the time of infection. In this case series we compare normal and abnormal findings in the fetus, placenta, and selected maternal tissues from ongoing infectious disease studies with both early pregnancy loss and full-term infants.

## Reducing the Burden of MRSA Colonization in Research Macaques

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Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a significant problem for human and animal health and affects the health status of nonhuman primate (NHP) colonies and research. In 2015 we sought to determine the MRSA carrier prevalence, risk factors associated with carrier status, and genotypes of MRSA within a diversely sourced population of research NHPs. The MRSA colonization rate was 28% (82/282), with strong risk factors being the room location and age of the animal. Based on these results, several critical changes to the facility's veterinary and husbandry practices were instituted including: 1) Improved antibiotic stewardship through eliminating standard antibiotic treatment of all animals entering facility and eliminating perioperative antibiotic usage on many animals undergoing basic surgical procedures, 2) Stricter workflow and room entry restrictions; and, 3) Enhanced PPE requirements within the animal facility. In the summer of 2018, we repeated the MRSA colonization survey within the facility to determine the impact of these changes. Our preliminary results show a striking reduction in the MRSA cocolonization rate to 9% (20/222). In addition, 48% (108/222) of the animals are colonized with *Staphylococcus aureus* displaying susceptibility to a broad array of antibiotics. This is in stark contrast to the 2015 survey, where a broad resistance pattern to antibiotics was more common. Thus, it appears that improved antimicrobial stewardship and management practices are associated with marked declines in MRSA prevalence and antimicrobial resistance in NHPs.

## Practical Strategies and Refinements in Postoperative NHPs with Cranial Implants

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Cranial implants, including head-posts, recording chambers, and microelectrode arrays are commonly used in nonhuman primates for neurophysiological research. Due to the nature of these long-term studies, these animals are frequently maintained on a study for years and will likely undergo multiple repair surgeries. Cranial implant refinements have helped reduce the number of repair surgeries in NHPs, but they have not eliminated them entirely. Aside from the common postoperative challenges such as infection and implant rejection, NHPs can easily access these incision sites and implants, hence impeding the healing process. Distracting NHPs from investigating their incision sites and implants can be extremely challenging, especially with their dexterous abilities. Traditional methods such as jackets and medications are an option; however, they can lead to negative effects on the animal's behavior or interfere with neurologic recordings. Other options involve implementing various forms of enrichment such as social housing, play cages, movies, foraging boards/boxes, etc. At our institution, the majority of our NHPs are enrolled in neurophysiological studies and almost all of them

have various forms of cranial implants. Occasionally, we come across NHPs who are notorious and relentless at removing sutures, and manipulating newly placed implants. Despite our enrichment efforts, these animals continue to investigate their head. To combat these situations, we have instituted the use of customizable and detachable caps to fit over the incision site or cranial implant. This novel and simple approach does not require the use of anesthesia or sedatives, and has decreased the number of postoperative complications associated with curious NHPs. In addition to improved animal welfare, i.e. normal appetite, unrestricted movement, and enhanced healing, most animals are able to return to their study sooner. This report illustrates the practical methods used to construct these detachable caps and how they positively impact animal health and the success of research procedures.

## When the Tuberculin Skin Test Fails: One Institution's Experience with Identification of TB in Research Cynomolgus Macaques

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While the mandatory screening by administration of intradermal tuberculin skin test (TST) in captive nonhuman primate colonies has resulted in significant reduction of *Mycobacterium tuberculosis* (*M. tb*) incidence, animals imported from countries with high rates of human infection still present a significant risk to research institutes and open colonies utilizing them. At a research institution in Maryland, U.S., cynomolgus macaques were identified with *M. tb*. Upon discovery of the first macaque with *M. tb*, room and building level quarantines, changes in personal protective equipment, consideration and employment of various diagnostic screening methods, retrospective post-mortem lesion analysis, and enhanced employee tuberculosis (TB) screening were conducted. A test-and-cull strategy was established for cynomolgus macaques housed in the facility utilizing standard TSTs paired with serology conducted with a commercially available simian TB test. Antemortem serology yielded 6/120 animals positive for *M. tb* complex (*M. tb*, *M. bovis*, *M. kansasii*). All macaques that were euthanized had full necropsies, histopathologic evaluation, and PCR testing of tissue. Of the 6 animals that tested positive, 1 had significant gross lesions that were positive on histopathologic evaluation, PCR and culture for *M. tb*. Post-mortem serology using stored samples revealed an additional 1/10 animals positive for *M. tb* complex. A retrospective survey of histopathologic evaluations of animals necropsied within that last 2 y revealed an additional 6 animals with granulomatous lesions and when tested for *M. tb* with immunofluorescent staining were positive. TST failed to identify any positive animals in the colony at any time. Improved TB surveillance, beyond TST monitoring, should be considered for high risk groups of nonhuman primates. Incorporating serology as a component of a TB screening program for cynomolgus macaques may prevent the loss of animals, disruption of research, occupational health risk to personnel, and overall economic burden related to outbreak and disease control.

### **Fluconazole Feed Management for Coccidioidomycosis (Valley Fever) in a Breeding Colony of Pigtail Macaques**

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Coccidioidomycosis (Valley Fever) is an endemic issue that needs management in the Southwest for humans and nonhuman primates. The management of Coccidiomycosis is evolving in both groups. Much of the concern is based on the issues of exposure and understanding the cyclic nature of these exposures and minimizing them. The other aspect of this issue is diagnosis and management of exposed animals. The gold standard for pharmaceutical treatment is fluconazole. A practical approach (due to initial number of animals and resource allocations) was to initiate fluconazole feed management for animals that have titers indicative of infections. This practice was initiated 2-y-ago at this facility. We have selected a subgroup of individuals on treatment and are evaluating the effectiveness of this approach through serum titer responses (IgG and IgM) and corresponding serum levels of fluconazole in these animals. The animals have responded well (based on titer responses, physical conditioning scores, and semiannual physicals) in a fairly rapid time period. One animal out of 9 had been on fluconazole for 60+ d and did not achieve therapeutic fluconazole serum levels, but has shown titer responses. The history of these animals will be presented as well as their titer responses and serum fluconazole levels. The discovery of some of the management issues with the feed (palatability and concerns when regular food is offered, environmental cleaning, secondary dental findings, reintroduction concerns with animals that have been temporarily removed, male breeders on medicated food that have been introduced to females not on the diet) will be discussed and reviewed. We are also in process of doing a comparison of feed management verses individual treatment management to better evaluate these outcomes. The current program management direction as well as the future overall management of the concern will be outlined.

### **Blood Pressure Reference Intervals for Ketamine-sedated Rhesus Macaques (*Macaca mulatta*)**

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Appropriate calculation and use of reference intervals has widespread clinical and research implications. Unfortunately, reference intervals for blood pressure in one of the most commonly used nonhuman primate species, the rhesus macaque (*Macaca mulatta*), have never been calculated. While anesthetic drugs and noninvasive methods of blood pressure measurement both have known effects on blood pressure values, their use provides the safest, fastest, and most widely used approach to clinical evaluation and blood pressure collection in this species. Noninvasive blood pressure measurements were collected from more than 100 healthy, ketamine-sedated, adult rhesus macaques, representing both sexes, aged 8 to 16-y-old, of varying body condition scores, with 2 types of sphygmomanometers, and at 3 different anatomic locations. Reference intervals were calculated for each device, in each location, establishing normative data beneficial to clinical veterinarians assessing animal health, and encouraging researchers to utilize

noninvasive methods. Age, body condition score, sex, type of sphygmomanometer, and location of cuff placement were all found to significantly influence BP measurements, providing important information necessary for the appropriate interpretation of noninvasive blood pressure values in rhesus macaques.

### **A Refinement to a Minimally Invasive Surgical Model for Intrathecal Catheterization and Ovariectomy in *Cynomolgus* Macaques Translatable to Tau Pathology Model**

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Induction of tauopathy in primates via intrathecal delivery of oligomer is being pursued to help with the mechanistic understanding of the pathology of Alzheimer's disease. The development of such an animal model represents a significant advancement for our scientists. Traditional access to the intrathecal space for catheterization required a major surgery that involving dissection of neck or back muscles and drilling a hole through a vertebral body. This model also required an ovariectomy in order to eliminate the estrogen source for optimal tracer signaling during translational imaging like MRI, PET etc. This was previously performed through a standard celiotomy involving a large abdominal incision. Due to the invasiveness of these 2 major surgeries, animals required significant amounts of analgesia and generally experienced prolonged, postsurgical recoveries. As a refinement, a minimally invasive percutaneous approach was established to catheterize the intrathecal space using a Touhy needle to act as a conduit for the catheter passage. The catheter is then connected to a port placed under the skin through a 1-2 cm incision. Similarly, the ovariectomy surgical procedure was reduced to a key-hole surgery using laparoscopic technique. Eleven nonhuman primates have undergone surgery using these refined techniques and all animals had faster recovery (i.e., normal food intake, body posturing, etc.) with less postoperative pain and complications than when using previous, more invasive surgical methods. All authors are employees of AbbVie. The design, study conduct, and financial support for this research was provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

### **MagPlex NHP MFIA: A Next Generation Multiplexed Fluorometric Immunoassay (MFIA) for Serodiagnosis of Nonhuman Primates Infectious Diseases**

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Multiplex immunoassays including Multiplexed Fluorometric immunoassay (MFIA) based on Luminex polystyrene (PS) beads have been in use for more than 15 y for routine serosurveillance of nonhuman primate (NHP) colonies. A new NHP MFIA using the next generation magnetic MagPlex microspheres was developed. MagPlex beads advantages over PS beads include no prefiltration of samples, no leaky expensive filter plates, and improved washing efficiency (quick and easy to separate from solution using a magnet). Antigens for several common infectious agents in NHP colonies including SIV, SRV, STLV, measles,

and B-virus were part of the 19 member NHP MFIA bead panel. Whole virus or purified recombinant antigens were individually coupled to different color coded bead sets. In addition, several system and sample suitability controls including tissue control beads to determine the sample related nonspecific antibody binding, species specific IgG and anti-IgG beads, were added to validate individual MFIA runs. Efficacy of this next generation MagPlex MFIA was compared to PS MFIA in a validation study using 16 known positive sera (for 1 or more infectious agents) from naturally or experimentally infected cynomolgus or rhesus. A similar number, 16 known negative macaque sera were used from specific pathogen free colonies. All samples were tested by 2 different technicians on 3 different days for a total of 6 runs. A total of more than 3000 assays were performed and analytical performance of the rodent MagPlex MFIA assay including selectivity and limit of detection was found to be comparable to or better than those obtained by PS MFIA. Overall diagnostic sensitivity of NHP MagPlex MFIA was 99% compared to 100% for PS MFIA. Diagnostic specificity of both NHP MagPlex and PS MFIA were nearly 100% suggesting that MagPlex MFIA is an acceptable alternative assay for serodiagnosis of adventitious infectious agents of NHP colonies.

### **Low-starch and High-fiber Biscuit Diet Reduced Obesity of Geriatric Japanese Macaques**

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Obesity can be difficult to control when laboratory animals are housed and fed ad libitum together in 1 large enclosure. Semi-annual body condition scores revealed geriatric Japanese macaques (*Macaca fuscata*) were overweight for many years in a large captive outdoor-housed troop. The standard fiber-balanced biscuits fed to the troop contained 26% starch and 12% fiber. In a trial to reduce obesity, a biscuit diet containing 8% starch and 18% fiber was fed to the troop of about 200 Japanese macaques. We hypothesized a low-starch and high-fiber biscuit diet fed ad libitum would reduce average body condition scores of geriatric age macaques. Body condition scores were recorded semi-annually for 3 y before and 2 y during the diet trial. An interrupted time series regression study design was used to evaluate the effectiveness of the population-level health intervention. Average body condition scores of geriatric macaques were significantly lower while the troop was fed the low-starch and high-fiber biscuit diet, but the diet trial was discontinued after 1.5 y on the trial when the average body condition score of geriatric macaques was thin. The average body condition score of geriatric macaques recovered when the troop was fed the standard fiber-balanced monkey chow.

### **Facial Swelling in a Cynomolgus Macaque (*Macaca fascicularis*)**

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A naive, 4-y-old, 5 kg female cynomolgus macaque (*Macaca fascicularis*) was found to have a focal, spherical, firm, subcutaneous mass on the proximal aspect of the nasal bone slightly right of midline during routine semi-annual physical examination.

Radiographs of the skull did not identify abnormal pathology and CBC/Chemistry were within normal reference intervals. Initially it was thought to be a blockage in the nasolacrimal duct and several attempts to flush the duct were made and treatment with heat packs was initiated. After several weeks of heat therapy, it was decided that the mass was not responding and a more aggressive approach was initiated. Attempts to lance and drain the mass were unsuccessful, and aspiration of the contents revealed a slightly red, stringy, viscous material. In the cytologic evaluation of this material occasionally cells with neoplastic features and pinkish extracellular materials were noted, suggestive of mesenchymal cell neoplasia, most likely osteosarcoma or chondrosarcoma. Bloodwork continued to be within normal reference intervals and repeat radiographs did not show abnormalities. An MRI scan revealed a contrast enhancing, soft-tissue mass that was slightly smaller in volume than the right eyeball. It was determined that this NHP would not be a good candidate for future drug studies and the decision to euthanize was made. Necropsy revealed a focal 2 x 2 cm raised, spherical, semi-firm, tan nodule on the proximal aspect of the nasal bone slightly right of midline that was solid on cut-section. No further macroscopic lesions were observed and the mass did not appear to be invading into the nasal turbinates, orbit, or cranium. Histologic appearance of the mass revealed a disorganized, hypocellular matrix of collagen and spindle cells with the presence of blood vessels and chondrocytes, suggesting a tumor of mesenchymal cell origin. To our knowledge, this is the first report of a mesenchymal cell neoplasia in the nasal bone of a cynomolgus monkey.

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

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Common marmosets (*Callithrix jacchus*) are a new world primate species commonly used in biomedical research with a documented predisposition to a spectrum of gastrointestinal diseases. At our facility, noninvasive methods to evaluate the gastrointestinal tract are limited to radiography with or without the use of intra-oral barium. Recently, several group-housed marmosets developed vomiting and weight loss. Following necropsy confirmation of duodenal ulceration and perforation in several of these animals, a more sensitive noninvasive monitoring technique was desired. In collaboration with veterinary radiologists, a protocol for focused ultrasound assessment of the cranial abdomen of marmosets was developed. The result was a simplified technique that could be easily taught to staff veterinarians and a 6-item checklist for assessment of the peritoneal space, hepatobiliary system, and proximal gastrointestinal tract. This technique was incorporated into biannual health screenings of the marmoset colony, with particular attention paid to any symptomatic animals. A total of 38 animals were evaluated using this technique. Ultrasound findings of proximal duodenal abnormalities ranging from mucosal irregularity to deep ulceration were observed in 6 animals. Of those animals with duodenal ulceration, 1 also had evidence of duodenal perforation at the time of ultrasound examination. Affected animals were treated with antiulcer and antimicrobial therapy or euthanized as appropriate for each individual case. Necropsy confirmed the presence of duodenal ulceration and/or perforation.

ration in the animal with sonographic evidence of duodenal ulceration and perforation. Noninvasive diagnosis and early detection is essential for proper management and long-term follow-up of duodenal ulceration and perforation in common marmosets. This focused ultrasound technique is easy to incorporate with routine health assessments of a colony and normal ranges generated in this study will be clinically useful for further studies of gastrointestinal disease in this species. We expect the technique of focused cranial abdominal ultrasonography may be adapted for use in other nonhuman primate species.

#### **Necrotizing Enterocolitis in a Premature Rhesus Macaque**

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Necrotizing enterocolitis (NEC) is a medical and surgical emergency for up to 6% of very low birthweight human infants. Infants colonized with *Ureaplasma parvum* are at higher risk. NEC has been reported in premature nonhuman primates (NHPs), however we could find no published reports of successful treatment. We present a case and medical management of NEC in a rhesus macaque. The infant was delivered via c-section on gestation day 135 (term=165) following intra-amniotic infection with *U. parvum* as part of an IACUC approved study related to premature birth. Neonatal intensive care was initiated and included respiratory, fluid, and nutritional support. Fluids and total parenteral nutrition were administered via a peripherally inserted central catheter. Small volume enteral feedings began on day 1 with human breast milk and transitioned over the first postnatal week to cow's milk-based formula. On postnatal day 7, the infant was noted with increased abdominal circumference and concern of NEC. Abdominal radiographs revealed diffuse gaseous distension of the intestines and stool was positive for occult blood. Medical management for NEC was started and included discontinuation of enteral feedings, gastric emptying, and broad-spectrum antibiotics with ampicillin and gentamicin. Response to treatment was evaluated using serial abdominal radiographs along with monitoring of the infant's abdominal circumference, vitals, and activity. After 1 week of antibiotics and bowel rest, enteral feeding was again initiated with human breast milk. Stool was negative for occult blood 16 d after original presentation and the infant was transitioned to formula with no further clinical concerns. This is the first report of successful treatment of NEC in a premature rhesus macaque. Since NHPs are models of morbidities associated with human premature infants, it is important to monitor for NEC and develop treatment options associated with this disease in NHPs.

#### **Unexpected Death in a Common Marmoset (*Callithrix jacchus*)**

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A total of 18 common marmosets (*Callithrix jacchus*) were released from a quarantine facility after 30 d and transported to our animal facility for breeding. All the animals were physically checked and all appeared in good body condition upon receipt. Unexpectedly, one 1-y-old male marmoset was found dead in

his cage after 3 d in his new cage. This marmoset was housed together with another male marmoset that were previously housed together in the quarantine facility successfully without any aggressive behaviour. However aggressive behaviour between these 2 animals was seen a few hours after the transfer and on the following day they were housed separately in their own cage. Favorite foods were given and monitoring and observation were done during daily observation of animals. Upon presentation for necropsy, physical examination of the dead marmoset presented a site of bruising at the right elbow involving the skin and underlying muscles. Opening the abdominal cavity revealed a mottled colored liver. A sample of the liver was collected for culture. Primary findings on histopathology revealed that the lymph nodes and spleen were inflamed with characterizable intracellular bacteria. Gram stain from these organs shown a gram negative bacteria. Similar bacteria were also noted to be present within the blood vessel lumen of many organs. The liver contains severe hepatic lipidosis typically due to a catabolic state imbalance prior to death despite good nutritional status. The liver sample processed for culture came back positive with *Klebsiella pneumoniae*, a common inhabitants of the mucosal surfaces of mammals and the environment. They are considered opportunistic pathogens capable of causing disease when an animal is stressed or its defence mechanism is altered. The final diagnosis on this animal is septicemia due to trauma associated bacterial infiltration by *Klebsiella pneumoniae*. This report highlights the need to closely monitor marmosets subjected to a stressful episode (transportation and injury), which results in a drastic change in their behavior.

#### **Efficacy of Fresh Neem (*Azadirachta indica*) Leaves for Treating Baboons (*Papio hamadryas*) Naturally Infected with *Trichuris trichuria***

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*Trichuris trichuria* is a nematode parasite that infects the cecum and large intestine of humans as well as nonhuman primates and is often found in captive and wild baboons. Neem (*Azadirachta indica*) is a tree native to India and its leaves and oil have been evaluated as a gastrointestinal dewormer in certain livestock species with varying results. The present study investigates the use of neem leaves as a potential treatment for naturally acquired *Trichuris* infections in baboons (*Papio hamadryas*). Seventeen juvenile baboons ranging from low to high contaminators were treated with fenbendazole (50 mg/kg PO SID x 3 d, n=7), fresh neem leaves (3 g/kg PO SID x 5 d, n=7), or acted as a control (n=3). The subjects were singly-housed and fecal egg counts (FEC) were obtained using the modified McMaster technique. All baboons treated with fenbendazole stopped shedding by day 15 post-treatment. However, there was no significant decrease in FEC in the control group or those that received the neem leaves. The results of this study indicate that fresh neem leaves are not effective at eradicating *T. trichuria* in baboons.

#### **Retroperitoneal Cystic Teratoma in a Cynomolgus Macaque (*Macaca fascicularis*)**

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A 2-y-old, 2.1-kg female cynomolgus macaque (*Macaca fascicularis*) socially-housed in an outdoor same-sex peer group presented with an irregular soft tissue mass in the right cranial abdomen palpated during routine semiannual physical exam. Ultrasonography revealed a multilocular mass with compartments of hypoechoic (fluid) or mixed echogenicity contents. Multiple irregular heterogeneous (mineral) opacities within the cranial abdomen were observed on radiograph, and a barium series confirmed the mass was extragastrointestinal and suggested adjacent organ displacement. During an exploratory laparotomy, a large multiloculated cystic mass was adhered to the right dorsolateral peritoneum and diaphragm, compressing the lateral margin of the right kidney medially, and displacing the entire abdominal viscera to the left. The mass did not appear to be associated with either ovary. After aspiration of approximately 100 mL of clear straw-colored fluid from 1 compartment the mass for decompression and improved access, it was excised. The mass had an irregular surface, weighed 139 g, and measured 8 x 10 cm. The mass contents varied by compartment: clear straw yellow fluid, caseous white material mixed with clear yellow fluid, turbid yellow fluid with high viscosity, flat irregular cartilage- and bone-like structures, dry caseous white material with pieces of hair, and tissue resembling skin. Cytology of the turbid yellow fluid diagnosed a squamous-lined keratin-producing cystic mass; scant growth of a coagulase-negative *Staphylococcus* sp. was present after 72 h of culture from the same compartment. There are few reports of teratoma in cynomolgus macaques, but prevalence is likely higher than reported because smaller masses may not be detected grossly.

#### **Solitary Trichoepithelioma in a Squirrel Monkey (*Saimiri sciureus*)**

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A 20-y-old male squirrel monkey (*Saimiri sciureus*) presented for lethargy and respiratory difficulty. Physical exam revealed mild cyanosis with increased respiratory rate and effort. There was a large firm, lobulated, moveable subcutaneous mass present on the anterior aspect of the animal's neck, which appeared to be partially compressing his trachea. This monkey participated in neuropharmacology studies previously. Due to poor prognosis, the monkey was euthanized and submitted for necropsy. Gross necropsy exam revealed the left salivary gland was replaced with a well-circumscribed, smooth, encapsulated mass measuring 5 x 2 x 1 cm. Histology findings were consistent with a follicular tumor originating in the subcutis and extending deeply to obscure the salivary gland. Progressive maturation of basal, spinosal, and granulosa cells were present along with keratinization, features which are most consistent with a diagnosis of trichoepithelioma. Trichoepitheliomas are benign cutaneous tumors arising from proliferation of follicular germ cells. They are rare in both humans and nonhuman primates; there is only 1 report in the literature of a trichoepithelioma in a Barbary macaque (*Macaca sylvanus*). In humans, trichoepitheliomas may occur as a solitary non-familial form or as part of multiple familial forms, which are inherited as an autosomal dominant disorder. Solitary trichoepitheliomas can affect humans of any age and sex, but the mean age of presentation is reported to be 60 y. Diagnosis of trichoepithelioma is based on fine needle aspiration cytology or histology, and immunohistochemistry can be used to differentiate trichoepitheliomas from

other closely related skin cancers such as basal cell carcinoma. Treatment of solitary trichoepitheliomas can be achieved with surgical excision, though local recurrence is possible. To the authors' knowledge, this is the first reported case of a solitary trichoepithelioma in a New World nonhuman primate species.

#### **Congenital Hyperostosis with Angular Limb Deformity in a Preterm Rhesus Macaque (*Macaca mulatta*)**

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A preterm, female rhesus macaque was delivered at gestation day 141 via cesarean section for an IACUC-approved lung development study. Standard resuscitation with a combination of positive pressure ventilation and continuous positive airway pressure (CPAP) were initiated as part of the study. On physical examination, the thighs and upper arms were enlarged with increased diameter and firm on palpation. The legs appeared in a bow-legged (varus) alignment. The carpi and elbows were enlarged with a decreased range of motion. During CPAP, the macaque developed tachypnea, nasal flaring, mild cyanosis, and intercostal retractions consistent with respiratory distress syndrome. The lungs sounded harsh on auscultation with diffuse crackles. Radiographs revealed a diffuse, unstructured interstitial pulmonary pattern. Radiographs also demonstrated disruption of cortices and periosteal proliferation of the ulnas, tibias, femurs, and humeri, as well as linear mineral opacities adjacent to the humeral and femoral diaphyses. Due to a poor prognosis, the infant was euthanized. At necropsy, the lungs were diffusely edematous and atelectatic. The femurs and humeri were markedly thickened and expanded with abundant mineralized periosteal tissue which merged with and expanded surrounding soft tissue and skeletal muscle. Similar but milder changes were present in the forelimbs and lower legs. Aerobic and anaerobic cultures of the blood, lung, and femur were negative. Histologically, the femurs, humeri, ulnas, and tibias were hyperostotic with marked periosteal proliferation and neutrophilic and necrotizing inflammation. Microscopically, maxilla, nasal turbinates, and lumbar vertebrae showed similar but milder changes. This animal is 1 of 3 similarly affected, premature rhesus macaques born at the ONPRC; a heritable condition similar to infantile cortical hyperostosis (Caffey syndrome) in humans is suspected.

#### **Psoriasisiform Dermatitis in Rhesus Macaques**

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Dermatitis characterized by chronic ulceration, hyperkeratosis, and xerosis of the ischial callosities and the palmar and plantar surfaces of the hands and feet was diagnosed in 17 colony rhesus macaques (*Macaca mulatta*). Indoor housed males were preferentially affected and the age at diagnosis ranged from 3 to 10 y with an average onset of 6 y. A psoriasisiform pattern was evident on microscopic examination with acanthosis, rete ridge elongation, and parakeratotic hyperkeratosis. Other features were perivascular, pleocellular inflammatory infiltrates, and spongiosis. The differential diagnosis was allergic contact dermatitis and was favored given the lesion distribution and

the character of the inflammatory infiltrate. Pathogenesis was thought to involve subsequent exposure to an allergen following initial sensitization. Dermatitis is a common, often clinically frustrating disease to manage in nonhuman primates. Identification of the underlying etiology and topical treatment can be challenging. Treatment involves a 3-step approach: identification and removal of the allergen, reduction in inflammation, and restoration and protection of the skin barrier. Epicutaneous patch testing is the gold standard noninvasive method for identification of inciting allergens. Potential allergens are placed in patches on the back and removed in 48-72 h. The underlying skin is then observed for signs of irritation. Despite testing, an inciting antigen has not yet been identified in our colony. Treatment of inflammation can be achieved using various strengths of immune modulating drugs. First line of treatment involves topical corticosteroids including triamcinolone and hydrocortisone creams. Oral corticosteroids are reserved for lesions covering greater than 20% of the body. Restoration of the skin barrier can be achieved through topical application of barrier creams or petroleum based emollients. The greatest success in treating animals at our institution has been achieved through elimination of contact with wet caging, topical or oral corticosteroids, and cage-side application of barrier cream to affected areas.

#### **A Variant STLV-1 Isolated from A Cambodian Origin Cynomolgus Monkey (*Macaca fascicularis*)**

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Simian T-lymphotropic viruses (STLVs) belong to the genus *Deltaretrovirus* and family *Retroviridae*. Members of STLVs together with phylogenetic closely related human T-lymphotropic viruses (HTLVs) are referred as primate T-lymphotropic viruses (PTLVs). STLV infect many Old World monkey species and great apes. Five types of STLV (STLV-1, 2, 3, 4, 6) have been identified and a highly divergent STLV-1 (MarB43) found in *Macaca arctoides* is provisionally named as STLV-5. STLV-1 has been distributed in Asia and Africa, but current knowledge of the diversity and prevalence of STLV-1 in different Asian primates is still limited. Here we report the isolation and complete genome of a STLV-1 variant strain (STLV-1/TX/2017) from a captive Cambodian origin cynomolgus monkey. Phylogenetic analysis of complete genome showed that STLV-1/TX/2017 strain formed a divergent lineage within the PTLV-1 group and was separated from STLV-2, 3, 4, 5 clusters. Significant lymphoid hyperplasia had been observed in multiple tissues of the variant STLV-1 infected animal. Serological survey of STLV-1 positive cynomolgus monkeys originated in Cambodia showed that at least 2 different serological patterns were present, suggesting that multiple STLV-1 lineages may exist in Asian cynomolgus monkeys.

#### **Pole and Collar Training of Chlorocebus Monkeys for Ophthalmic Study Purposes**

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At our preclinical research facility, pole and collar training is used for macaques to facilitate handling, especially in combi-

nation with chair restraint for dosing or procedures. Animals undergo a period of training to acclimate them to having a pole introduced into their cage and having it clipped to their collar. Animals are then trained to exit the cage for study purposes such as sitting in a restraint chair. Pole and collar training allows technicians to safely handle macaques that are too large for hand catch restraint and can minimize the use of chemical restraint for handling. This voluntary participation with positive reinforcement may lead to overall stress reduction for the animals. We were faced with the challenge of conducting an ocular study using adult Chlorocebus monkeys. These animals were not accustomed to being hand caught and manually restrained, and many of them were too large to be safely restrained manually. In order to minimize animal stress and the need for chemical restraint, we decided to pole and collar train the Chlorocebus monkeys so that we could use restraint chairs for routine study procedures. The pole and collar training process for Chlorocebus monkeys was very different than our normal pole and collar training procedure in macaques. Major differences included longer training time needed for Chlorocebus to bond with handlers and the stoic nature of the Chlorocebus compared to macaques. We were ultimately able to successfully use pole and collar handling for these animals on study, which facilitated day to day study activities, as well as veterinary assessment and care. Our experience shows that this technique is applicable to Chlorocebus monkeys, but requires the training of personnel to adapt the training procedure and be flexible to the needs of the animals.

#### **Use of Automated Feeders to Detect Clinical Inappetence in Socially-housed Rhesus Macaques (*Macaca mulatta*)**

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Reduced food intake is a clinical sign for many diseases in nonhuman primates (NHPs). Inappetence can also occur early in disease development, prior to the onset of weight loss or more serious symptoms. Individual food intake quantification, however, is difficult in group-housed NHPs and often requires temporary removal of animals from their social group. Thus, noninvasive methods to identify animals with acute or gradual changes in appetite would be beneficial to NHP veterinarians. Automated feeders are commercially available that reliably record individual calorie consumption in rhesus macaques; however, little information is available regarding the timing and degree of calorie intake reduction associated with common clinical conditions in this species. Currently, the Yerkes NPRC uses automated feeders to provision food to several large outdoor breeding groups of rhesus macaques. Here, we report changes in -24 h kcal intake associated with cases of trauma (n=320), diarrhea (n=196), and fetal loss (n=36) from these groups over the past 3 y. Although food intake from automated-feeder fed animals is monitored daily, feeding data for this study were analyzed retrospectively according to subject age and sex. Analyses revealed that trauma and fetal loss cases were associated with acute decreases in kcal intake ( $P < 0.05$ , -24 h vs. -30 d-avg. baseline values), whereas diarrhea cases were associated with more gradual decreases in kcal intake ( $P < 0.001$ , -24 h and -7 d-avg. vs. -30 d-avg. baseline values). Among the trauma cases, adult female crush trauma and male juvenile/yearling digit trauma with bone exposure had the greatest mean percent decreases in kcal intake at -24 h. Diarrhea cases with infectious

etiologies showed greater decreases in kcal intake at -24 h than nonpathogenic cases. Because small fluctuations in daily intake is expected in healthy monkeys, these analyses will help veterinarians interpret automated feeder-generated feeding reports and guide decisions regarding when to remove inappetent animals from their group for examination.

### **Use of Automated Feeders to Monitor Food Intake Prior to Trauma Incidence among Groups of Socially-stable Rhesus Macaques**

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Automated feeders can be used to assess individual feeding patterns in socially-housed monkeys; however, little information is available regarding the usefulness of this system in the management of group stability. We previously reported that the targeted animals of an intra-family fighting incident within a large outdoor breeding group of rhesus macaques at our institution exhibited reduced food intake prior to the onset of significant fighting. Additionally, the percent decrease in kcal intake from baseline to -24 h in targeted animals significantly predicted the severity of their wounds. These findings suggested that injured animals may have anticipated contact aggression from group mates and had stress-induced inappetence as a result. It is unknown, however, whether these findings translate to individual trauma incidents occurring during periods of social stability. Thus, we report here changes in daily kcal intake associated with treated and nontreated trauma cases from 3 established outdoor breeding groups of rhesus macaques. A severity sum score was calculated for all acute trauma cases ( $n=296$ ) based on the number of trauma incidents each animal received during the 60-d baseline period. We found that animals temporarily removed from their social group for trauma treatment, compared to non-treated cases, exhibited a greater percent decrease in kcal intake from baseline to -24 h ( $P < 0.001$ ). Group means of percent change in kcal intake for treated and nontreated adults and juveniles were negative; however, only yearlings with treated traumas exhibited a significant decrease in kcal intake (-52%) at -24 h ( $P < 0.01$  compared to baseline). Similar to findings from our case study of intra-family fighting, a greater percent decrease in food intake at -24 h significantly predicted higher trauma severity sum score ( $P < 0.001$ ,  $\beta = -0.169$ ). These findings suggest that automated feeder-generated feeding data can be used successfully in the management of colony social health, particularly when analyzed in parallel with trauma and social dominance data.

### **Root Cause Analysis: Harnessing Human Variability to Avert Errors**

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Like all highly reliable systems, animal husbandry relies on a system of standard operating procedures, formal training, and continual monitoring to ensure animal welfare. Each of these layers has inherent vulnerabilities which, when aligned, may lead to undesirable outcomes. A staff member misreads a diet label, and provides the wrong research diet to assigned

study animals. A new cage design is introduced, and untrained personnel improperly secure a slide, latch, or lock. A technician, looking for a safe, clean space to store medication syringes while doing other injections in the room, places them on the light timer case, forgets to retrieve one, and an animal misses an essential injection. Rarely are these events intentional. Organizational response can focus on the fallible human component, relying on a culture of shame and blame to prevent further occurrence. Highly reliable industries, aviation and health care, employ a systems approach to identifying and managing these risks. We present 1 technique, root cause analysis, for system focused error prevention. This process includes a rigorous evaluation of all factors which contributed to the event, provides a clear and formal process for identifying, implementing, and evaluating solutions, and allows standardization for tracking and trending. More importantly, by actively engaging people in the analysis process, we foster a culture of continual system improvement with proactive identification of and response to system vulnerabilities.

### **Single Photon Emission Computed Tomography-computed Tomography (SPECT-CT) Imaging with Technetium-99m Labeled Leukocytes as a Novel Tool for Evaluation of Cranial Implant-associated Infection in a Rhesus Macaque (*Macaca mulatta*)**

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An adult male rhesus macaque (*Macaca mulatta*) enrolled in a study evaluating cognition and memory presented with suppurative exudate along the margins of a long-standing cranial implant that included a stainless steel head post, left side recording cylinder, and acrylic over a previously located right side recording cylinder. Both cylinders were located at the level of the prefrontal cortex. Following management of the implant with systemic antibiotics and daily cleaning with betadine for several months, the macaque underwent single photon emission computed tomography-computed tomography (SPECT-CT) imaging in which his neutrophils were labeled with technetium-99m-hexamethylpropylene amine oxime ( $^{99m}\text{Tc-HMPAO}$ ) to evaluate for active infection below the implant. Soft tissue inflammation and osteomyelitis were found at the site of the previous right-sided recording cylinder. A cephalosporin and tetracycline antibiotic were administered for 12 wk. Follow-up SPECT-CT imaging was then performed to evaluate response to medical treatment and results indicated no change in the degree of soft tissue inflammation or osteomyelitis associated with the right side recording cylinder site. SPECT-CT imaging was used to guide surgical removal of the implant and debridement of the infected tissue. On removal of the entire cranial implant, the osteomyelitis and soft tissue inflammation observed on both SPECT-CT scans were confirmed. Additionally, a large cavitory defect through the calvarium with suppurative exudate was discovered below the base of the head post. Infection in this defect was not apparent on SPECT, however the defect in the bone was subsequently confirmed on re-evaluation of the CT images. It was concluded that the infection in this defect was silent on SPECT due to limited vascularization of the surrounding bone. This case study is the first report describing use of SPECT-CT imaging for evaluation of soft tissue inflammation and osteomyelitis beneath a cranial implant in a nonhuman primate.

## Alterations in Hematology and Microbiology of Chronically Implanted Rhesus Macaques

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Rhesus macaques are used in neuroscience research due to similarities to humans in neuroanatomy, physiology, social behavior, and cognition. Studies in brain function often require direct access to cortical structures for visualization, stimulation, or deactivation, and this is achieved through the use of cranial implants that provide ports to specific brain structures. The presence of an implant can become a nidus for chronic infection, affecting hematologic values and complicating the assessment of blood work. The purpose of this study was to determine how cranial implants affect hematologic values in clinically healthy rhesus macaques used in neuroscience research as well as identify the pathogens that cause infections in these animals. Using our clinical pathology database, we compiled data on complete blood cell counts and cultures from implants on 57 clinically healthy rhesus macaques from 3 different neuroscience research groups at our institution from 2002 to 2017. Using a repeated measures generalized linear mixed model, we analyzed the difference in hematology and clinical chemistry values between non-implanted and implanted monkeys. The target values were hematocrit, neutrophils, lymphocytes, macrophages, basophils, and eosinophils, and age and research group were added as fixed effects to control for these variables in all models. Analysis revealed that rhesus macaques with implants had significantly lower hematocrit, lymphocyte, eosinophil, and basophil counts, and higher neutrophil counts than those without implants. Research group and age were statistically significant in all models. Of the 93 aerobic implant cultures submitted, *Staphylococcus* species were found most frequently on culture at 73.1%, followed by *Streptococcus* (22.6%), *Corynebacterium* (20.4%), and *Enterococcus* (14.0%) species. *Fusobacterium* was the most frequently cultured anaerobic bacteria at 13%. This study demonstrates that cranially implanted animals are physiologically affected by the presence of their implants despite showing no clinical signs, and that care should be taken when analyzing CBC results.

## Pharmacokinetics of a Long-acting, Highly Concentrated Buprenorphine Solution (Simbadol™) in Rhesus Macaques (*Macaca mullata*) after Subcutaneous Administration

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Opioids are essential for rhesus macaques (*Macaca mulatta*) requiring multimodal analgesia or those unable to receive NSAIDs as part of their pain management plan. The current opioid epidemic has universally limited the availability of these vital analgesics compelling clinicians to investigate other options including novel opioid formulations. A long-acting, highly concentrated formulation of buprenorphine, is a single dose, injectable solution that provides therapeutic plasma concentrations lasting 24 h in cats (*Felis catus*). We hypothesized that this highly concentrated buprenorphine solution (HCBS) would achieve therapeutic concentrations ( $\approx 0.1$  ng/mL) for

at least 24 h in rhesus macaques. The objective of this study was to evaluate the pharmacokinetic profile of a single subcutaneous dose of HCBS in rhesus macaques at 0.24 and 0.72 mg/kg and then compare them to each other and the cat. Six healthy, adult rhesus macaques were included in a randomized, 2-period, 2-treatment crossover study. Plasma buprenorphine metabolite concentrations were determined prior to and for a maximum of 120 h after administration, measured using liquid chromatography-tandem mass spectrometry and pharmacokinetic analysis was performed. The low dose achieved a maximum plasma concentration of  $19.1 \pm 5.68$  ng/mL at  $19.6 \pm 4.02$  h with an AUC of  $236.4 \pm 22.5$  h/ng/mL and a terminal elimination half-life of  $19.6 \pm 4.02$  h; for the high dose, these parameters were  $65.2 \pm 14.7$  ng/mL at  $0.034 \pm 0.004$  h,  $641.3 \pm 79.4$  h/ng/mL, and  $20.6 \pm 2.30$  h, respectively. The mean concentration at 48 h postinjection was significantly ( $P < 0.01$ ) above the therapeutic threshold for both dosages in macaques. One animal showed mild pruritus at both doses while another showed mild somnolence at both doses. These findings support the use of HCBS in rhesus macaques for once every other day dosing without problematic adverse effects and represent a potential new alternative.

## Comparing Methods for Integrating Adult Male Rhesus Macaques (*Macaca mulatta*) into Group Housing

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Integration of adult male rhesus macaques into new social groups is essential in maintaining a successful breeding colony. Current practice at our institution includes 2 types of male integration: 1) new animals are introduced into a group of other conscious macaques or 2) all animals are placed in an enclosure while anesthetized. Practices also vary with respect to how a new breeding group is established, i.e. males added to existing all-female groups (MtF) or other methods (OTHER), including introduction of females into bachelor groups or group formations involving strangers. Regardless of integration or group type, males often must be removed due to social trauma. We hypothesized that allowing animals to recover from anesthesia together would result in a higher percentage of adult male monkeys remaining within the group. Data were collected from 253 adult male social integrations in large field enclosures (>5000 sq. ft.) conducted at the institution from 2015-2018. In addition to type of integration (conscious vs. anesthetized), data were analyzed by age, number of adult males introduced, number of breeding aged females in the group, pattern of trauma (male vs. female), and group type (MtF vs. OTHER). Outcome was measured at 14 and 30 d following integration. Fourteen days post integration, 59% of anesthetized integrations were successful compared to 38% of conscious integrations, but this was not statistically significant ( $P = 0.07$ ). When comparing group types, MtF groups were less successful than OTHER groups (14 d:  $P = 0.011$ , 30 d:  $P = 0.003$ ). Male-pattern trauma was not associated with any independent variables but female-pattern trauma was significantly more common in MtF groups ( $P = 0.007$ ). Although our original hypothesis was not supported, this study identifies other variables that influence integration success and may allow for refinement of future practices.