Journal of the American Association for Laboratory Animal Science
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Vol 57, No 1
January 2018
Pages 70–84

Abstracts of Scientific Papers

2017 Association of Primate Veterinarians Workshop

Characterization of the Microbiota Colonizing Cephalic Recording Chambers of Rhesus Macaques (*Macaca mulatta*)

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Macaques (*Macaca mulatta*) are the most commonly used nonhuman primate in cognitive neuroscience research due to similarities between the macaque and human brain. Cephalic recording chambers (CRC) are often surgically implanted to obtain neuronal recordings. CRCs represent a persistent source of microbial contamination, which can occasionally progress to clinical sequelae of meningitis and cephalic abscess. Although characterization of aerobic bacterial species in CRCs by culture-based methods has previously been documented, limited-to-no information is available on fastidious aerobic and anaerobic flora colonizing CRCs. We hypothesized that analysis of the CRC microbiota would help elucidate the diversity of bacteria inhabiting CRCs. Purulent CRC exudate was sampled using a sterile syringe from 9 CRCs in 7 rhesus macaques. Genomic DNA was extracted using a commerical DNA microbiome kit. The V4 region of the 16S rRNA gene was amplified by PCR, and sequenced using 250 paired-end reads by a commercial vendor. Microbiota analysis was performed using commercial software. Principal coordinate analysis of weighted Unifrac distances revealed unique microbiota profiles in all 7 animals, with no apparent subgrouping based on age, sex, clinical status, duration of implantation, or investigator laboratory. For macaques with dual implants, 1 macaque showed a similar microbiota profile between the 2 CRCs while the other macaque had differing microbiota profiles between the 2 CRCs. *Fusobacterium* spp. was detected in 6/7 macaques and contributed >26% of sample reads in 5 macaques. The order *Clostridiales* accounted for >54% and >70% abundance in the 2 CRCs from the macaque without *Fusobacterium* spp. Common oral flora, including *staphylococcus*, proteus, bacteroides, *peptostreptococcus*, and *prevotella* were noted to contribute >1% of sample reads in a majority of macaques. Operational taxonomic units common to all 7 animals included the *Clostridiales* and *Bacteroidales* orders, the *Ruminococcaceae* family, and the *Shewanella*, *Halomonas*, and *Staphylococcus* genera. Our results demonstrate the feasibility of acquiring microbiota data from purulent exudate, and highlight the contribution of anaerobic species to the CRC microbiota.

Six Cases of Surgical Removal Of Enormous Cranial Implants in Rhesus Macaques (*Macaca mulatta)*

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Nonhuman primates with cranial implants are frequently used in neuroscience research. However, limited information exists in the scientific literature and textbooks to guide in their surgical removal. In this case series, chronic cranial implants were surgically removed from 6 male rhesus macaques (*Macaca mulatta)*. This was performed either following study completion in preparation for retirement or due to chronic refractory infection of the implant. The implant base was composed of a plastic ring mounted to the skull with 6 screws surrounded by dental acrylic. The implant's design promoted granulation tissue formation around the margins and in the interface between the skull and overlying acrylic. These areas became prone to bacterial infection, including infection involving MRSA. Also, the extensive use of acrylic promoted lysis of underlying bone. The procedure consisted of chip-wise removal of plastic and acrylic components from the skull using wire cutters, rongeurs, and periosteal elevators. Screws were then removed using a dental drill and granulation tissue was scraped away using dry gauze. The margins and exposed skull were then flushed with saline and antibiotic-soaked gauze was applied for approximately 10 min (3 songs). The area was then aseptically prepared using standard techniques and surgeons donned sterile attire. Margins were freshened and extensively undermined. Various techniques to promote skin apposition, such as scoring of underlying tissue, V-Y plasty, meshing, and advancement flaps were used in a patient-dependent manner. Postoperative care included frequent incisional cleaning, broad-spectrum antibiotics, analgesics, and laser therapy as needed. Removal was successful in all 6 cases with minimal complications.

Unusual Presentation of a Chronic Brain Abscess in a Rhesus Macaque (*Macaca mulatta*)

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A 7-y-old, intact, male rhesus macaque (*Macaca mulatta*)with a cranial implant of 4 y duration was found acutely down following a 6 mo history of short, intermittent lethargic episodes. Bloodwork analysis and physical exam revealed no abnormalities, yet the animal became progressively worse over the next 2 d. An MRI of the head was performed and a large radiopaque area in the dorsal surface of the right hemisphere of the brain was observed, along with a right-to-left midline shift in the brain. A preliminary diagnosis of abscess was made. Treatment options were discussed, but given the severity of the MRI findings and the clinical signs, euthanasia was elected. A full necropsy was performed, and an abscess was confirmed to be present on the right dorsal surface of the brain. *Staphylococcus aureus* was cultured from the abscess. The parietal bone over the right hemisphere was approximately twice as thick as the left, causing a right-to-left midline shift of the brain. We hypothesized that chronic inflammation due to the presence of the abscess resulted in hypertrophy of the adjacent cranial bone over the course of several months. The intermittent clinical signs displayed by this animal were considered to be due to this progressive expansion of the lesion, and a subsequent increase in intracranial pressure to which the animal adjusted. With time, the extent of the inflammatory bone expansion and increased pressure resulted in the animal's inability to compensate.

Development of an Allogeneic Hematopoietic Stem Cell Transplant Model in Mauritian-Origin Cynomolgus Macaques (*Macaca fascicularis*)

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Animal models of HIV/AIDS have provided a foundation for our current understanding of HIV pathogenesis, treatment, prevention,

and more recently, curative strategies. The first and only case of a functional HIV cure was reported in 2009, when patient Timothy Brown, referred to as the "Berlin patient," showed no signs of HIV following an allogeneic hematopoietic stem cell transplantation (HSCT) for leukemia using a graft derived from a ccr5Δ32/Δ32 donor. Unfortunately, attempts to cure other HIV-infected patients through HSCT have failed, thus highlighting the need for animal models to understand how the immunologic responses associated with HSCT and graft-versus-host-disease (GvHD) can be safely harnessed for the elimination of viral reservoirs in HIV-infected patients. Mauritian cynomolgus macaques (*Macaca fascicularis*, MCM) are the ideal candidates for this model because they are an insular population that resulted from a small number of founder animals over the last 500 y and have exceptionally low genetic diversity relative to other macaque species, thereby allowing the identification of fully MHC-matched HSC donor-recipient pairs. This presentation will review the rationale for using MCM in this HSCT model, the HSCT procedure and associated clinical care as it is performed our institution, and the range of clinical outcomes of HSC transplanted MCM. To date, we have performed 10 HSCT in MCM with clinical outcomes comparable to those observed in human HSCT. The outcomes have ranged from graft failure/rejection, acute and chronic GvHD, opportunistic infections postHSCT, and long-term stable HSC recipients with high-level (up to 100%) donor chimerism. Ultimately this preclinical model will further our understanding of allogeneic HSCT-induced GvHD, both how it can be harnessed to purge latent HIV from the body, and how to disentangle the protective graft-versus-tumor effect from the detrimental side effects of GvHD to improve clinical care of both HIV and HSCT patients.

Hydrocephalus as a Consequence of Intrathecal Administration of Dextran in Rhesus Macaques (*Macaca mulatta*)

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Dextran has been used extensively as a medical therapy and labeling agent in biomedical research to investigate the blood brain barrier and cerebrospinal fluid flow and absorption. Adverse effects from the use of dextrans have been reported to include anaphylactic reaction and dilation of the cerebral ventricles due to administration into the subarachnoid space. This retrospective study evaluates the use of dextran administered intrathecally and the resulting hydrocephalic state demonstrated in numerous rhesus macaques (*Macaca mulatta*).

Eosinophilic Aortitis with Severe Aortic Dissecting Aneurysm and Rupture in a Captive-Born Owl Monkey (*Aotus trivirgatus*)

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A 5-y-old, captive-born, male, research naïve owl monkey (*Aotus trivirgatus*)was found dead during morning rounds. The animal was in good body condition, weighed 800 g, and had a history of heart murmur. At necropsy, the left hemithorax contained a large blood clot while the right hemithorax contained serosanguineous fluid. The entire length of the cranial aorta out of the heart to the aortic hiatus in the diaphragm was dilated and irregularly shaped with its widest dimension being approximately 1.3 cm. Approximately, 1 cm proximal to the diaphragm, a 5 mm diameter tear of the aorta was noted. No other gross lesions were observed. Histologically, the aorta had marked intimal thickening due to accumulation of mucinous ground substance, loss of the elastic lamina, separation of the tunica media by a space filled with blood, and an outer band composed of abundant granulation tissue, fibroplasia, edema, mucinous deposits, and moderate infiltrate of eosinophils. The outer layer contained multifocal areas of necrotic cells admixed with degenerate neutrophils. The myocardium had multiple coronary arteries and arterioles vacuolated with necrotic myocytes in the tunica media. A few larger arteries had mild-to-moderate eosinophilic infiltrate surrounding them. The kidney had multifocal mild-to-moderate interstitial lymphoeosinophilic infiltrates, foci of interstitial fibrosis, multiple degenerate cortical tubules, and attenuated epithelium filled with proteinaceous fluid, eosinophilic granular cast material, or neutrophils or foamy macrophages. The liver had diffuse mild-to-moderate hepatocellular hemosiderosis. The cause of death in this animal was severe thoracic aortic aneurysm with dissection and rupture causing peracute exsanguination into the pleural cavity. The final diagnosis was eosinophilic aortitis with severe aortic dissecting aneurysm and rupture. Interestingly, the pathology findings resemble features of Wegener's granulomatosis and Churg-Strauss disease, both human diseases that are part of a complex group of autoimmune large vessel vasculitides.

Refining the Perioperative Critical Care of Nonhuman Primates Undergoing Solid Organ Transplantation

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Approximately 55,000 people are currently waiting on organ transplant lists, many of whom will die without having the chance to receive a potentially life-saving organ. In addition, organ transplantation is associated with high comorbidities such as infection, immune-disorders, and lifelong immunosuppressive drugs, putting a huge burden on both the patient and health care system. Thus, organ transplantation continues to be a much needed area for research, with improvements having the potential for significant impact to human health and welfare. One way to overcome these limitations is to induce long-term immune tolerance in recipients. Nonhuman primates continue to serve as a critical model for understanding the immunobiology of acceptance, rejection, and tolerance of allogenic and xenogenic transplantation. The nature of organ transplant research is inherently invasive, and it is the responsibility of the institutional animal care program to continually refine and advance the standard of medical care. Of critical importance for model success is the provision of optimized perioperative intensive care. Here, through a series of cases, I will outline the development of a novel tolerance model for liver transplant in the cynomolgus macaque, and the significant advances we have made in the preoperative, intraoperative, and postoperative care of these animals.

Rhesus Macaques (*Macaca mulatta*) with Familial Left Ventricular Hypertrophy and Sudden Cardiac Death Serve as a Nonhuman Primate Model of Hypertrophic Cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is an inherited disease that leads to arrhythmias, congestive heart failure, and sudden death in humans. Although understanding of genetic and phenotypic aspects of HCM has advanced significantly in recent years, it has not yet led to practical information for the prevention or treatment of HCM. Naturally occurring left ventricular hypertrophy (LVH) with sudden death has been identified in rhesus macaques (*Macaca mulatta*). We hypothesize that familial LVH in rhesus macaques may serve as a naturally occurring, nonhuman primate model of HCM

based upon phenotypic and genotypic similarities to human HCM. Our objective was to identify sarcomeric mutations that may explain a rhesus macaque model of HCM through extensive phenotyping and whole exome sequencing. LVH was diagnosed at necropsy when the ratio of the LV diameter to the LV luminal diameter was greater than 3 in. a transversely sectioned heart. DNA from 65 LVH-affected rhesus macaques was used to investigate possible genetic factors contributing to disease. Whole exome sequencing was performed using the custom Rhexome rhesus macaque exome capture reagent and the data mined for sarcomeric variants. One hundred and thirty-three randomly selected rhesus macaques served as the control population for case:control association analysis. The whole exome sequencing identified multiple variants in sarcomeric genes. We found that 46 of the 65 LVH-affected rhesus macaques (71%) carried at least 1 missense mutation in the sarcomeric gene myosin heavy chain 7 (MYH7). This frequency is dramatically elevated relative to controls ($P < 2 \times 10\text{-}11$). All the variant genotypes were heterozygous, consistent with observations that dominant mutations in MYH7 can cause human HCM. A nonhuman primate model of HCM is being developed. These animals share clinical, pathologic, and genetic similarities with the human disease and represent an exciting area for future research into this devastating disease.

FDG/PET Kinetic Modeling for Evaluation Pathophysiological Alterations after Ebolavirus Infection

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In the aftermath of the 2014 ebolavirus outbreak in West Africa, major efforts have been undertaken to develop innovative imaging methods for studying pathogenesis in animal models. The majority of deaths in human patients and animals are the results of organ failure and shock brought on by the uncontrolled release of cytokines. Alterations in organs and tissue functions can be assessed by clinical imaging which is used in the monitoring, staging, and treatment of human patients. FDG-PET imaging is a molecular imaging technique that is available for accurate quantitative assessment of cellular metabolism in the whole body. It is also useful to noninvasively detect and quantify inflammation, infection, and other etiologies of altered tissue metabolism in organs of the body. During infection, activated inflammatory cells use glucose as a source of energy resulting in high FDG accumulation at sites of infection and inflammation. Infection is also characterized by alteration in tissue perfusion that can be detected by the changes in FDG delivery to affected sites by using tracer kinetic analysis of dynamic FDG-PET data. By analyzing a complete sequence of images acquired from the time of FDG injection dynamic FDG-PET can separate cellular response from vascular processes, allowing us to better visualize pathogenic processes in nonhuman primate models of ebolavirus disease. The presentation will provide the data on variability and reproducibility of kinetic constants in lymphoid tissue and in the liver of normal NHPs.

In Vitro and In Vivo Attempts to Infect Baboons (*Papio* sp.) with Simian Betaretrovirus (SRV)

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Although there are no confirmed reports of an exogenous Simian betaretrovirus (SRV) isolated from baboon (*Papio* sp.) in the scientific literature, apparent antibody reactivity has been observed repeatedly in various colony animals. This finding confounds the management and utility of these animals for research studies. To provide further insight into this observation, we have initiated both in vitro and in vivo studies to determine if baboons can be infected with SRV. Previously we had isolated, stimulated, and cocultured peripheral blood mononuclear cells (PBMC) from 6 baboons demonstrating indeterminate or positive SRV antibody with known susceptible, uninfected macaque PBMCs, Raji, or SupT cells. No virus was detected, because although some mild cytopathologic effects were noted in a few cultures, all were negative for SRV1-5 DNA when tested by PCR for 8 wk. Recently, PBMCs from 2 SRV seronegative baboon donors were isolated, stimulated, and inoculated with SRV1 or SRV 2 tissue culture virus. The cultures were observed and tested positive by SRV1-5 PCR for 2 wk. To ensure that the virus detected was not residual input, the cells were washed and subcultured with fresh, uninfected baboon PBMCs for 2 additional weeks. SRV1 PCR remained positive for both donors and SRV2 for one. Further analysis of the cells and supernatants is in progress. The same SRV virus stocks are now being transfused into naïve baboon hosts to determine if in vivo infection will occur. In addition, transfusions of SRV-infected blood from donor macaques into other naive baboon hosts will be attempted. Blood samples are being collected for antibody testing and PCR to monitor for seroconversion and/or the presence of viral nucleic acid. The results of this study will likely aid in developing guidelines for the management and research use of captive baboon colonies.

Evaluation of Analgesic Patches in Cynomolgus Macaques (*Macaca fascicularis)*

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Opioids are a fundamental component of preemptive analgesia and multimodal pain management protocols used to minimize pain in laboratory animals. However, there is a paucity of information regarding the pharmacokinetics of opioid transdermal patches in nonhuman primates (NHPs), with only one study that analyzed the use of fentanyl patches, and none that characterize the use of buprenorphine patches. Therefore, opioid transdermal patch use in NHPs is often inferred from clinical application in humans or studies in other animal species. In an attempt to better manage painful conditions in NHPs, the pharmacokinetics of fentanyl (25mcg/hr) and buprenorphine (10 mcg/hr and 20 mcg/hr) transdermal patches were evaluated in healthy, young adult male cynomolgus macaques (*Macaca fascicularis)* (n=4) in a crossover study. Plasma opioid levels were determined by tandem liquid chromatography-mass spectrometry with blood samples collected at 0, 4, 8, 12, 24, 36, 48, 60, 72, 80, 96, 120, 144, 168 h, and 24 h postpatch removal. Noncompartmental pharmacokinetic analysis was performed using a commercially available pharmacokinetic software package. The elimination half-lives for fentanyl and the low and high dose buprenorphine patches were 21.71 ± 4.25, 76.59 ± 27.05, and 42.40 ± 10.62 hr, respectively. Maximum plasma concentrations for fentanyl and the low= and high-dose buprenorphine patches were 2.42 ± 0.61, 3.43 ± 1.18, and 8.07 ± 3.85 ng/mL, respectively, occurring at 14 ± 7, 57 ± 15, and 45 ± 6 hr. The areas under the curve for fentanyl and the low- and high-dose buprenorphine patches were 127.07 ± 7.16, 300.79 ± 102.22, and 678.51 ± 295.89 ng·hr/mL, respectively. No adverse effects were noted during or after patch application. Therapeutic concentrations for fentanyl (≥ 0.2 ng/ml) and buprenorphine (≥ 0.1 ng/mL) were extrapolated from human and canine data. Fentanyl plasma concentrations reached therapeutic levels in all 4 macaques within 4-8 h and were maintained for greater than 96 h postpatch application. Buprenorphine plasma concentrations for both the low and high doses reached therapeutic levels in all four macaques within 4-12 h and were maintained for 144-168 h. These findings suggest that 25 mcg/hr fentanyl patches should be replaced every 4-5 d while both the low and high dose buprenorphine patches would need to be replaced every 6-7 d. Understanding the

pharmacokinetics of commonly used opioid transdermal patches in nonhuman primate medicine will aid clinicians in making pain management decisions, ultimately improving animal welfare.

Refining Medication Administration with a Novel Dispensing Device

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Selection of drug regimens can be complicated in primate medicine due to the frequency of administration required. Many oral pharmacological agents must be administered every 8-12 h to ensure efficacy, limiting their practicality due to their short duration of action. As one example, trimethoprim sulfadiazine has activity against gram-negative and gram-positive bacteria and is a useful antibiotic for treating a myriad of ailments, from respiratory infections to abscesses. However, the recommended 3 times-a-day daily dosing renders the drug impractical for most nonhuman primate facilities without 24-h routine care capabilities. Such difficulties of administration limit the use of many similar drugs for nonhuman primates, leading to the use of a handful of drugs with longer dosing intervals even when they are less than ideal for a given problem. In order to offset the logistical complications of frequent administration and stress induced by injections, a novel device was constructed to allow for timed dosing throughout a 24-h period. A commercially available timed feeding device with 5 slots was purchased and a fiberglass case custom built to enclose it. The bottom of the case contains a small circular cut out that allows a primate to collect a food item, while preventing the animal from obtaining enough leverage to rotate the device prior to set times. The top of the case can be removed for daily loading of the device with food items that may contain medication. Once secured into the case, the device is hung horizontally on the outside of the cage with the access hole facing the animal. Once the device was constructed, a pigtail macaque requiring chronic treatment for diarrhea secondary to amyloid deposition was selected to test the utility of the device. In order to habituate the animal, the device was mounted to the outside of her cage with the timed rotation feature deactivated. When accustomed to the device's presence, the rotating feature was activated. Counterconditioning was used in the form of enrichment in each slot to encourage the primate to explore the device following the sound generated by rotation. Once enrichment was consistently taken, medication was introduced into enrichment in 3 of the slots, the remaining slots contained enrichment only. Each morning the device was removed to verify medication consumption. The animal of interest was treated for 14 d using the device and reliably retrieved enrichment and consumed the incorporated medication.

Ethical and Veterinary Oversight Of Primate Sanctuaries: Multilimb Amputation in a Ringtail Lemur (*Lemur catta*), A Case Report

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There is significant interest in developing suitable sanctuaries for nonhuman primates, whether animals are being retired from research, entertainment use, or being relinquished from private ownership. One of the most challenging aspects is ensuring appropriate and knowledgeable veterinary support is available for routine and emergency veterinary care. The cadaver of a 6-y-old intact male ringtail lemur *(Lemur catta)*originating from a private exotic animal sanctuary was submitted for postmortem examination. A quarantine had been imposed at the sanctuary by the regional public health office following identification of sanctuary animals positive for *Echinococcus multilocularis*, and the collection of nonhuman primates, including several lemurs, a squirrel monkey, and a Japanese macaque, were moved into the sanctuary owners' house and cohoused uncaged during the day with supervision. Shortly afterwards and almost 1 mo prior to submission, this lemur suffered marked trauma following an attack by the macaque, resulting in severe bite wounds, skin loss, tail mutilation, and bilateral forelimb fractures. The forelimb fractures had initially been corrected with pinning and plating at a nearby companion animal clinic. The tail, both hind legs, and the right fore arm were subsequently amputated successively over several weeks at the same clinic. The owners had refused euthanasia and the lemur had been treated with antibiotics and meloxicam during this interval but it died spontaneously 3 d following the last surgery. Upon gross examination, the animal presented with bilateral hindlimb, right forelimb and tail amputations, in addition to having an open fracture of the left ulna, deep purulent ulcers over the sacrum, as well as purulent material draining from the leg stump suture sites. Ringtail lemurs are semi-arborial/terrestrial species and use their tail and all limbs for normal ambulation. The ethical, veterinary care, and welfare implications of this case will be presented, in addition to reporting follow-up.

Weight Loss and Neurological Deficits in an Adult, Female Rhesus Macaque (*Macaca mulatta*)

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A 13-y-old Indian-origin, female rhesus macaque (*Macaca mulatta*) presented for weight loss, circling, poor hand-eye-coordination, proprioceptive deficits, and persistent lactation despite a 2 y history without confirmed pregnancy. Complete blood count, chemistry panel, and urinalysis were unremarkable. A team of board-certified ophthalmologists performed a nonsedated visual assessment evaluating pupillary light reflexes, proprioception, and response to the optokinetic drum and a flashing light. A sedated retinal examination was performed, and the optic nerve appeared subjectively pale. One wk after the initial ophthalmic exam, another exam was performed and results were compared to an age and sex matched control which confirmed optic nerve pallor in the affected animal. Magnetic resonance imaging demonstrated a large, lobulated hypophyseal mass with an associated mass effect, most consistent with a pituitary macroadenoma. Blood was collected to determine serum thiamine, estrogen (E2), progesterone (P4), and prolactin levels. Thiamine, estrogen, and progesterone levels were within normal limits; however, prolactin was significantly elevated. Open room behavior and activity observations revealed repetitive counterclockwise perimeter circling, as well as an inability to direct gaze at food or occupational enrichment placed in the immediate walking path. Given the animal's poor prognosis, euthanasia was elected. An expansive, compressive pituitary adenoma was diagnosed on necropsy. Immunohistochemistry staining and electron microscopy were performed. Interestingly, this animal demonstrated clinical signs of hyperprolactinemia (galactorrhea and elevated serum prolactin levels) despite tumor cells having negative immunoreactivity for prolactin. We propose that hyperprolactinemia in this case is due to stalk syndrome or pituitary stalk compression syndrome, a phenomenon in which nonsecretory suprasellar tumors induce hyperprolactinemia by inhibiting dopamine delivery to lactotrophs. Since dopamine is a prolactin-inhibiting factor, reduction of dopamine levels leads to increased prolactin output. It is hypothesized that dopamine levels are reduced by 1 of 2 mechanisms: 1) physical compression of the dopaminergic neurons of the infundibular stalk; or 2) disruption of hypophyseal portal blood flow which delivers dopamine to lactotrophs.

Chronic Progressive Cerebellar Ataxia and Visual Impairment in Japanese Macaques (*Macaca fuscata*)

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Five Japanese macaques (*Macaca fuscata*) from the same colony presented over a 5-y period with varying degrees of abnormal gait, decreased coordination, intention tremors, and hypermetria. One animal also presented with obvious partial visual deficits. In all of the subjects, these symptoms presented between 4-5 y of age, gradually progressing over time, with all animals requiring euthanasia before 6 y of age. Evaluation with multimodal retinal imaging and multifocal electroretinography revealed decreased retinal thickness, increased autofluorescence, and reduced retinal response amplitude with foveal sparing. MRI examination revealed signs of cerebral and cerebellar atrophy which were confirmed on gross examination at necropsy, in addition to diffuse cerebral and cerebellar tan discoloration. Histopathologic examination of the central nervous system and retina revealed widespread degenerative changes with atrophy of cerebellar folia, Purkinje cell loss and thinning of the granular cell layer, and tan granular material within neurons that autofluoresced and was further characterized on electron microscopy as granular osmiophilic deposits. These findings are all consistent with a diagnosis of neuronal ceroid lipofuscinosis, a genetic lysosomal storage disease, with this being the first documented model of NCL in a nonhuman primate species. Pedigree analysis and whole exome gene sequencing discovered a cluster of variants in the CLN7/MFDSD8 gene including a single base deletion predicted to cause loss protein function. Segregation of the CLN7 variant is consistent with an autosomal recessive mode of inheritance. Presymptomatic homozygous recessive animals in the colony are now identified along with the heterozygous carriers. The shared characteristics of Japanese macaque NCL with CLN7 NCL disease in humans makes this an invaluable opportunity for studying this disease and developing potential therapeutic interventions.

Lameness in a Juvenile Rhesus Macaque (*Macaca mulatta*)

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An approximately 5-y-old male captive bred rhesus macaque (*Macaca mulatta*) was pair-housed at an academic research institution as part of a postirradiation fertility study. After a routine anesthetic event, lameness was noted in the right leg. Physical exam showed only mild intermittent crepitus on palpation of the right knee joint and moderate muscle atrophy of the right leg. Radiographs were taken, and radiology consult noted flattening of the right femoral head which is consistent with avascular necrosis. Osteopenia of the right femur, tibia, and fibula, as well as diffuse right lower extremity muscle atrophy were also noted. Nonsteroidal antiinflammatory (NSAID) treatment was initiated and continued for 2 wks with no improvement in lameness noted. There was no change in lameness or clinical condition after NSAID treatment was discontinued. After scheduled euthanasia for the research protocol, a necropsy was performed. Necropsy showed deformation and flattening of the right femoral head, marked erosion and loss of the cartilage of articular surface of femoral head and acetabular cavity, loss of epiphyseal growth plates of femoral head, and osteopenia of the cortical and trabecular bone of the entire right femur. In addition, the right hip joint had marked thickening of the joint capsule and rupture of the round ligament of femoral head. Histopathology findings confirmed necrosis of bone, bone marrow, and cartilage of the right femoral head. Necropsy and histopathology findings, along with the clinical findings and radiographic observations, were consistent with the diagnosis of Legg-Calvé-Perthes (LCPD). LCPD is necrosis of the femoral head and neck, caused by impairment of the blood supply to the femoral epiphysis. No definitive cause of this disease is known. There are limited cases of Legg-Calvé-Perthes disease in nonhuman primates described in the literature.

An Unusual Finding in a Chagas-Positive Rhesus Macaque (*Macaca mulatta*)

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A 20-y-old Chagas-positive male rhesus macaque (*Macaca mulatta)* presented for weight loss and lethargy. Physical exam revealed hypothermia, mild dehydration, decreased and resonate cardiac sounds, and abdominal distention characterized by a fluid wave, hepatomegaly, and multiple small, firm nodules and fibrous bands extending from the xiphoid to the pubis. Serum chemistry revealed hypoproteinemia, hypoalbuminemia, elevated BUN, and elevated liver enzymes. Hematology revealed a moderate lymphopenia. Differential diagnoses included ascites and hepatomegaly secondary to cardiac dysfunction, Chagas-related cardiomyopathy, or neoplasia. Subcutaneous normal saline and enalapril were administered. The following day, an intravenous catheter was placed and diphenhydramine was administered prior to administration of albumin mixed with normal saline IV and subcutaneous fluids. During recovery from anesthesia, the heart rate decreased and the macaque was not recovering. Euthanasia by intravenous barbiturate overdose was elected and a complete necropsy was performed. The abdomen contained over 500 mLl of transudate fluid. The abdominal cavity caudal to the liver was covered in thickened, nodular, firm omentum adhered to the surrounding organs and abdominal wall. A thick fibrous band extended from the omentum to the caudal abdominal wall. In the midbody of the stomach was a depressed dark red to black friable area in the mucosa surrounded by a firm, thickened gastric wall. The corresponding serosa was continuous with the nodular omentum. Mesenteric lymph nodes were markedly enlarged. The gastric mass was consistent with gastric adenocarcimona with hemorrhage, desmoplasia, suppurative inflammation, and transmural invasion. Metastatic lesions were found in the omentum, liver, capsular surface of the spleen, mesenteric lymph nodes, and serosal surfaces of the intestines and urinary bladder. Warthin Starry stain of the stomach revealed *Helicobacter* organisms in the mucosa.

Evaluation of Zinc Gluconate as a Nonsurgical Sterilization Method In Rhesus Macaques (*Macaca mulatta*)

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It is widely known that rhesus macaques (*Macaca mulatta*) are prolific breeders in captivity and in the wild. Therefore, overpopulation can be problematic in research and feral populations. Currently, the most effective contraceptive methods are hormonal control in female macaques and vasectomies in males. These methods each come with their own innate challenges, foremost being the alteration of necessary hormonal maintenance. In this study, we assessed the use of zinc gluconate as a novel, nonsurgical alternative to male contraception. Zinc gluconate was given as a 1-time, intratesticular injection to cause permanent infertility while theoretically sparing the testosterone producing Leydig cells of the testis. Complete blood counts, serum biochemistry analyses, testosterone levels, and testicular widths were evaluated at the time of injection and either 1 wk, 1 mo, 2 mo, or 3 mo postinjection. Daily post injection observations noted transient scrotal swelling in most animals but no indications of pain. Additionally, full necropsies including

testicular histopathology were assessed at study endpoints. The majority of animals had normal testicular architecture and sperm production up to 3 mo postinjection. In conclusion, chemical castration with zinc gluconate is not an effective sterilization method in rhesus macaques.

Use of Automated Feeders to Monitor Group Stability in Captive Breeding Colonies of Rhesus Macaques (*Macaca mulatta*)

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Captive breeding colonies of rhesus macaques (*Macaca mulatta*) commonly live in large social groups comprised of multiple matrilines. Fighting within the same matrilineal family or between different families due to social instability can result in trauma and mortality. Thus, a primary goal in the management of these groups is the collection of behavioral and clinical data to determine the presence of social unrest before the onset of significant fighting. Changes in social dominance and the frequency of trauma are commonly used to monitor group stability. However, psychologic stress has been associated with food intake reduction in other animal species; therefore, inappetence in key individuals or groups of monkeys could be used as another indicator of emerging instability. An incident of intrafamily fighting occurred recently involving the 5th-ranked family of a large breeding group (n=126, 8 families) at our institution that resulted in 4 cases of rhabdomyolysis or moderate female-inflicted trauma and 6 cases of male-inflicted trauma. Because this compound was equipped with automated feeders that quantify individual calorie intake, feeding data was analyzed retrospectively to determine if any significant reduction in daily kcal consumption occurred prior to onset of fighting and baseline values (previous 30-d average). No significant differences in total kcal intake for the whole group and individual families were observed between baseline and previous 24 h values; however, the affected 5th-ranked family (n=16 adult females) exhibited a nearly significant ~20% reduction in total kcal intake from baseline to previous 24 h ($P = 0.06$). Most notably, the targeted subfamily (n=4 adult females) showed a marked ~57% reduction in food intake in the 24 h prior to the fighting incident ($P < 0.01$), while the remaining subfamilies showed no significant changes in appetite. The alpha and beta females, as well as the alpha male, also exhibited marked decreases in food intake during the same period. These findings indicate that automated feeders can assist management staff with monitoring group stability in rhesus macaque breeding colonies.

Daily Water Intake of Common Marmosets (*Callithrix jacchus*) Used in Biomedical Research

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The typical daily water intake of common marmosets (*Callithrix jacchus*) in a research setting has not been well characterized. Because these new world primates are increasingly popular as animal models in neurophysiological and behavioral experiments, which can include the potential use of water regulation for training, veterinary and research staff need to understand how marmosets normally regulate their hydration status. A study was undertaken to measure the water consumption of older (5-12-y-old, n=11) and younger (1-2-y-old, n=10) adult marmosets every 3 h during the 12-h light cycle (7 a.m. to 7 p.m.) for the months of January and July. The results show the average water intake per animal was 38.71 ml/kg/day with a wide range across animals (minimum: 14.57, maximum: 129.40 ml/kg/day); however, water intake by an individual marmoset was fairly consistent from day to day. Water intake did not vary across the 4, 3-h periods measured during the day, and minimal water was consumed overnight when the room lights were turned off. Also, there was no significant difference in daily water intake between the two months ($P = 0.41$). A moderate but significant correlation ($R = 0.65$; $P < 0.01$) was found between age and water consumption, where older adults tended to drink more than the younger group. Singly housed marmosets drank more on a per kilogram basis than pair-housed marmosets ($P < 0.01$). Importantly, the considerable variation in water consumption among marmosets emphasizes the need for an individualization of fluid regulation protocols.

Analysis of Risk Factors for Endometriosis in Rhesus Macaques (*Macaca Mulatta*)

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Endometriosis is a chronic, progressive disease of menstruating primates characterized by dysmenorrhea, infertility, and chronic pelvic pain. To date there are few reports that characterize risk factors for development of endometriosis in nonhuman primates. We investigated potential reproductive risk factors that may contribute to endometriosis in 2 well-characterized research populations of rhesus macaques (*Macaca mulatta*). Four reproductive surgical techniques (hysterotomy, amniocentesis, ovarian follicle aspiration, and embryo transfer) were investigated via a retrospective case-control study of 466 females presenting to the pathology service of 2 national primate research centers from 2000-2014.Additional reproductive risk factors we investigated included family history of endometriosis, parity, maternal age at first and last parturition, age at which surgical techniques were performed, and presence of obstetric abnormalities (early- and late-term pregnancy loss, retained placenta, or dystocia). Follicle aspiration or embryo transfer did not significantly increase the risk of developing endometriosis. Similarly, maternal age at first parturition, parity, and nulliparity were not significant risk factors. Conditional logistic regression demonstrated a significant increase in the odds of developing endometriosis with hysterotomy (odds ratio, OR = 1.78; 95% confidence interval, CI = 1.41 — 2.24). Additional risk factors that were predictive of disease development included amniocentesis (OR = 2.19, CI = 0.92 — 5.24), total number of reproductive surgical procedures (OR = 1.125, CI = 1.02 — 1.24), affected first- or second-degree relatives (OR = 1.45, CI = 0.90 — 2.33), a history of 1 or more spontaneous early pregnancy losses (OR = 2.71, CI = 1.23 — 5.97) or a history of 1 or more retained placentas (OR = 2.33, CI = 0.89 — 6.12).

Hemorrhagic Cystitis in Rhesus Macaques (*Macaca mulatta*): Clinical Management and Colony Epidemiology

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A 9-y-old, male, Indian-origin rhesus macaque (*Macaca mulatta*) presented for frank hematuria and abdominal splinting. Urinalysis and culture revealed evidence of sterile hematuria and proteinuria. Abdominal ultrasound demonstrated moderate thickening of the bladder wall and no evidence of urolithiasis. Resolution of clinical signs occurred within 1 wk after treatment with antiinflammatories and analgesics. One month later gross hematuria returned with a more pronounced thickening and well-differentiated layering of the bladder wall. Abdominal radiographs were performed and had no evidence of urolithiasis or sperm cystolithiasis. Based on the progression of clinical signs, an exploratory laparotomy with bladder biopsy was elected. The serosal surface of the bladder was

engorged, with tortuous vessels and an overall increased level of vascularization. The wall was thickened and the mucosa appeared edematous with small cystic structures. Histologically the bladder wall was moderately hyperplastic with multifocally eroded urothelium. There was evidence of submucosal edema, eosinophilic and lymphocytic aggregates, neovascularization, and congestion. These findings were consistent with the diagnosis of idiopathic hemorrhagic cystitis. In our colony, hemorrhagic cystitis is often observed during potentially stressful events, such as quarantine or times of intense research protocol activity. Our average age at presentation is 8.3 y and notably, all of the affected animals have been male. Clinical presentation includes intermittent gross hematuria with negative culture and an average bladder wall thickness of 0.5 cm. Similar disease has been reported in both human and feline medicine, but there is minimal published information in the nonhuman primate literature. We present a representative case of hemorrhagic cystitis as well as epidemiologic data obtained from our institution's colony.

Development of a Macaque (*Macaca mulatta)* Model of Rectal Syphilis

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Men who have sex with men are at higher risk for acquiring HIV if coinfected with syphilis or other sexually transmitted infections (STI), but the effect of rectal STIs on biomedical interventions for HIV, like preexposure prophylaxis (PrEP), is unknown. A nonhuman primate (NHP) model of rectal syphilis in rhesus macaques (*Macaca mulatta)* was developed to study interventions for syphilis, as well as PrEP efficacy against simian/human immunodeficiency virus (SHIV). We administered *Treponema pallidum* submucosally (Nichols strain; up to 4 injection sites; 200-500µL volume; up to 10^8 live organisms/mL) in the rectum of 2 SHIV-infected rhesus macaques with a 29-gauge needle approximately 5 cm beyond the anorectal opening and monitored the resulting lesions by endoscopy. An additional 2 SHIV-infected rhesus macaques were inoculated intradermally as positive controls. PCR and dark field microscopy were used to detect *T. pallidum* in rectal tissue and blood. Treponemal assays (*Treponema pallidum* particle agglutination assay, TP-PA; syphilis screening tests) as well as nontreponemal methods evaluated serum antibodies. Rectal lesions were first observed as early as day 3 postchallenge and serum antibodies were TP-PA positive 5-6 wk after inoculation. Antibody levels peaked at 1:20,480 by week 14 before reaching a plateau. Syphilis assays detected antibodies as early as week 5. *T. pallidum* DNA was detected in blood at week 11 for 1 animal, indicating active syphilis infection. RPR has been nonreactive. We have successfully developed the first NHP model for rectal syphilis. This new model is being further refined and has applications for use in the context of other STI coinfections and antiretroviral drug efficacy studies.

Spontaneous Pulmonary And Disseminated Coccidioidomycosis in a Colony of Specific Pathogen-Free Pig-Tailed Macaques (*Macaca nemestrina*)

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Coccidioides spp. are dimorphic, soil-dwelling fungi, which are endemic in the arid desert regions of the southwestern United States and Mexico. Coccidioidomycosis has been documented in a wide variety of mammalian species, as well as some reptiles. Among nonhuman primates, spontaneous coccidioidomycosis has been documented in rhesus macaques (*Macaca mulatta*), bonnet macaques (*M. radiate*), Japanese macaques (*M. fuscata*), baboons (*Papio* spp.), Chimpanzees (*Pan troglodytes*) and a ring-tailed lemur (*Lemur catta*). From October 2011 to December 2016, coccidioidomycosis was diagnosed in 65 pig-tailed macaques (*M. nemestrina*), with gross and histologic confirmation, housed in indoor-outdoor facilities in Texas and Arizona. The most common clinical signs were weight loss and diarrhea. Other common clinical findings include respiratory signs such as coughing, hypothermia, the presence of cutaneous draining tracts, and acute decompensation with spontaneous death. Thirty-five of these macaques were evaluated for *Coccidioides* spp. specific antibodies, and had a positive titer for *Coccidioides* spp. Fungal spherules were identified in 5 of these macaques on cytology. Culture of 1 animal identified *Coccidiodes immitus*. The most frequent gross pathologic findings include hilar lymphadenopathy with pyogranulomas, pyogranulomatous pneumonia, and frequent dissemination. The most frequent histopathologic findings include pyogranulomatous lymphadenitis and pneumonia with fungal organisms, and frequent dissemination to distant organs. Additional common, potential secondary, findings include gastroenterocolitis and amyloidosis. The emergence of *Coccidioides* spp. as a significant pathogen in pig-tailed macaques is likely related to severe drought conditions, beginning in 2010 in the southwestern United States, and susceptibility of the host. Favorable environmental conditions allow the fungi to proliferate, and increase airborne exposure of the resident macaque population. The veterinary staff have implemented multiple management changes in response to these infections, including surveillance with serologic testing, moving animals to indoor HEPA-filtered air housing, and the use of fluconazol- impregnated feed.

Nonreducible Inguinal Hernia Containing Uterus and Bilateral Adnexa in a Rhesus Macaque (*Macaca mulatta*)

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A 3-y-old, captive-reared, female, Indian-origin rhesus macaque (*Macaca mulatta*) presented for preassignment physical examination. The patient had a history of mild splenomegaly and lymphadenomegaly. During physical examination, a firm, immobile subcutaneous mass was palpated in the left inguinal region. Uterine bimanual palpation revealed a 90-degree leftward turn between the cervix and the uterine body. There was no evidence of peripheral lymph node enlargement and the patient's CBC and serum chemistry were also unremarkable. An abdominal and inguinal ultrasound exam was performed. The uterine body was normal in size and echogenicity with a clearly defined hyperechoic endometrial stripe. However, the organ was displaced from its normal location. The uterus was herniated through the inguinal ring and corresponded to the externally palpable mass. To confirm the diagnosis and correct the hernia, the patient was scheduled for a herniorrhaphy. A linear incision, parallel to the long axis of the leg, was made over the herniated tissue in the left inguinal region, with subsequent dissection to and penetration of the vaginalis. All reproductive organs including the uterus, ovaries, oviducts, and fimbria, along with a small portion of the omentum, were present in the hernia sac. The organs and omentum were manually reduced into the pelvic cavity. The thickened left round ligament was partially transected to allow tissue replacement. A single, nonabsorbable cruciate suture was used to partially reduce the internal inguinal ring. This case represents the first report of indirect inguinal herniation of the uterus and adnexa in a rhesus macaque. While uterine inguinal herniation is a rare condition most often seen in infant humans, it can present in subadult macaques without any associated clinical signs and minimal observable abnormalities. As such, uterine inguinal herniation should be considered as a differential diagnosis in animals with unilateral subcutaneous enlargements in the inguinal region.

An Approach to Spontaneous Cardiopulmonary Arrest in Nonhuman Primates

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Spontaneous cardiopulmonary arrest is a rare but serious potential problem in nonhuman primate research colonies. Although primates have been used (albeit infrequently) as models of resuscitative therapy following induced arrest, occasionally veterinary personnel are required to respond to an unexpected spontaneous cardiopulmonary arrest. Animals undergoing sedation or anesthesia are at higher risk for adverse events, given that the agents used to achieve sedation and anesthesia have the ability to depress pulmonary and/or cardiac functions. Nonhuman primates are often sedated for routine procedures, such as physical examinations, and therefore have a higher risk for adverse events than other species that do not have to be sedated for handling. We have devised a coherent, straightforward flow chart detailing the sequence of events that should take place following an arrest, based on recommendations from the Veterinary Emergency and Critical Care Society RECOVER documents and human pediatric guidelines. The flow chart is included in all rooms that have the greatest potential for a spontaneous cardiopulmonary arrest, such as areas in which primates are sedated for physical exams, surgical preparation rooms, and operating rooms. We have also developed a standard operating procedure for training research and veterinary staff to respond immediately and efficiently following a cardiac arrest, as well as charts with relevant emergency drug dosages for old world as well as new world species. The goal of this management initiative is to decrease morbidity and mortality of primates undergoing sedation or anesthesia for routine procedures by implementing protocols to achieve a fast and efficient response in the event of a cardiopulmonary arrest.

Lockjaw due to Severe Degenerative Joint Disease in a Rhesus Macaque (Macaca mulatta)

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A naïve, 8-year-old, female rhesus macaque was on an eye movement study. On 11/7/16 she was sedated for surgery. We were unable to intubate, due to severe decreased range of motion of the temporomandibular joint (TMJ). There was no indication of illness prior to surgery (stable weight). Radiographs and blood work were unremarkable. A CT scan revealed severe degenerative joint disease of the TMJ. Degenerative joint disease is not uncommon in captive rhesus macaques, however this was an odd presentation. The animal was treated with steroids and supportive care.

A Novel Antimicrobial Treatment for Methicillin-Resistant *Staphylococcus aureus* in Nonhuman Primates

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Methicillin-resistant *Staphylococcus aureus* (MRSA), can cause both superficial and invasive disease and is responsible for millions of serious and sometimes fatal human infections worldwide. Currently, alternative antimicrobial therapies need to be developed due to the increase in antibiotic-resistant bacteria and the development of fewer new antibiotics. Lytic enzymes obtained from bacteriophages (lysins) and other bacteria (bacteriocins) are being studied as the source of potential novel antimicrobial agents. These enzymes can degrade the bacterial cell wall of their target species resulting in hypotonic lysis of the bacterium. Currently, a colony of nonhuman primates (NHPs, *Macaca mulatta*) with cranial implants tested positive for cutaneous MRSA despite standard decontamination protocols and antibiotic treatment. Besides concerns for animal health and zoonotic transmission, these animals could serve as MRSA infection models to test these lytic enzymes as a novel treatment therapy. A preliminary study using 1 bacteriocin treatment animal (lysostaphin 5 mg/mL) and 1 control animal showed that there was an immediate (0-3 d post treatment) 3 log reduction in the colony forming units (CFUs) after 2 consecutive daily topical treatments of the cranial implant margin compared to control. In brief, both animals and their environment underwent a 3 d decontamination protocol to reduce the risk of cross-contamination. Culture swabs were collected from their cranial implant margins during the decontamination, treatment, and posttreatment periods. Serial dilutions of the culture swabs were made and the number of CFUs was determined after 48 h of incubation at 37°C in MRSA selective chromogenic media. Unfortunately, 6 d after the second treatment the CFU count of the treatment animal increased and was similar to the control animal. Thus, we concluded that lysostaphin treatment alone was effective at reducing MRSA shortterm but not longterm. This may be explained by the risk of cross-contamination from untreated areas, the ability of the medication to reach all infected areas, inappropriate concentration and number of doses, and the need to combine different treatment modalities such as systemic and topical antibiotics and continuous skin/haircoat decontamination with antiseptic products to completely eradicate the pathogen. Thus, our objective for future studies is to conduct additional treatment trials to determine if the use of lytic enzymes in combination with other treatment modalities effectively eliminates MRSA infection/contamination in rhesus macaques. Results from these studies should prove useful in the development of novel treatment strategies for MRSA cutaneous infections.

Postural Tachycardia in a Rhesus Macaque (*Macaca mulatta*)

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An 8-y-old, 5 kg female Indian-origin rhesus macaque (*Macaca mulatta*) presented for tachycardia on routine physical exam. Heart rate exceeded 300 beats per minute (bpm) when the patient was seated upright under ketamine sedation; when the monkey was placed in lateral recumbency, her heart rate was 180 bpm. Routine bloodwork was within normal limits, and her SPO2 maintained at 100% while sedated. ECG revealed a sinus tachycardia with moderately elongated QRS complexes suggestive of mild left ventricular hypertrophy. Radiographs demonstrated a normal cardiac silhouette and cardiac dimensions. A human cardiologist was consulted, and echocardiography revealed no abnormalities in cardiac size, wall thickness, great vessels, or valve leaflets. Noninvasive blood pressure readings demonstrated a prolonged decrease in systolic blood pressure of 15-20 mm Hg after the patient was moved from a recumbent to a seated position. A tentative diagnosis of postural orthostatic tachycardia syndrome (POTS) was made based on postural tachycardia accompanied by prolonged orthostatic hypotension under sedation. Due to the risk of syncope as a sequela of this condition, which precluded long-term assignment to a social group or research protocol, euthanasia was elected. POTS is a dysautonomic condition most commonly diagnosed in women of reproductive age. It is characterized by tachycardia with or without hypotension, resulting in symptoms such as fatigue, dizziness, syncope, or discomfort. At necropsy, typhlocolitis was the prominent gross lesion. Generalized lymphangiectasia, epicardial and intestinal submucosal edema, villous blunting, and gastrointestinal lymphoid proliferation were apparent on histopathological examination. Although difficult to definitively diagnose, dysautonomic conditions such as POTS should be included as a differential when evaluating NHPs

with cardiac abnormalities in the absence of apparent antemortem pathologic findings.

Stifle Swelling and Lameness in a Juvenile Rhesus Macaque (*Macaca mulatta)*

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A 2-y-old, 2.7 kg male rhesus macaque (*Macaca mulatta)*presented acutely for right hindlimb lameness. The monkey was nonweight-bearing on the ipsilateral hindlimb in the social group and toe-touch lame on cageside evaluation. A 5 cm area of soft-tissue swelling encompassing the right stifle was apparent on intake evaluation. His lameness clinically improved with administration of meloxicam, tramadol, and cephalexin. However, a bilateral, 1 cm bony prominence distal to the stifles remained on recheck examination 7 d after presentation, and the monkey remained aversive to pressure applied at the right tibial tuberosity. Radiographs demonstrated increased bone density at the level of the tibial tuberosity with increased soft tissue radiopacity on the dorsal aspect of the tibia. Ultrasonographic evaluation of the right and left stifle joints demonstrated apparently normal menisci with mild periosteal elevation at the right tibial tuberosity. A diagnosis of apophysitis of the tibial tuberosity (Osgood-Schlatter disease) was made, and the monkey was given cage rest and meloxicam for an additional 7 d prior to release to the social group. On recheck exam 6 wk posttreatment, he demonstrated a normal gait with no muscular atrophy. Residual radiographic changes included bony proliferation at the tibial tuberosity with increased soft tissue radiopacity overlying the area. Osgood-Schlatter disease results from an overuse injury to the patellar tendon and tibial tuberosity in fast-growing adolescents, resulting in traction apophysitis at the tendon insertion site. Clinical signs in humans include knee pain centered over the tibial tuberosity that is exacerbated by athletic activity. It is generally self-limiting, though symptoms may wax and wane until bone maturation is complete. Treatment generally includes analgesia and physical therapy. This condition has not previously been reported in rhesus macaques; awareness of this syndrome may assist in more rapid treatment and recovery of affected individuals.

An Easy Microsampling Device for Routine Serosurveillance of Nonhuman Primate Colonies

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Traditional serology has been performed using serum and singleplex ELISAs. Multiplex ELISA techniques, including MFIA, require much smaller volume of serum, thus paving the way for use of microsampling techniques including dry whole blood spot (DBS), requiring only a single drop of blood. In this study, a new commercially available microsampler was optimized for routine serosurveillance of nonhuman primate colonies. The tips of the blood specimen collection device consist of inert, porous, hydrophilic material that quickly wicks up a consistent volume (20ul) of whole blood. The device has several advantages versus serum as they are quantitative, have improved reproducibility, and reduce animal stress due to easier sample collection. Qualification studies were conducted comparing analytical and diagnostic performance of paired microsampler and serum from rhesus and cynomolgus macaques. Matching sera samples (8 per species) from naturally and experimentally infected macaques (known positives) as well as from SPF macaques (known negatives) were tested. Study samples were tested by macaque MFIA assessment panel with 13 different assays. Average scores (analytical performance), titration curves, and limit of detection using polyspecific and monospecific sera were similar for microsample and serum. Diagnostic reproducibility and ruggedness was tested by 2 techs performing MFIA assays on 3 different d (6 runs total). Diagnostic sensitivity of microsample and serum was found to be 99.6% and 99.8% whereas diagnostic specificity was 98.6% and 99.6%, respectively. Average microsample scores and number of positives between the 2 analysts as well as microsampler versus serum were comparable with low CVs of around 10%. Qualification data demonstrates that the microsample is an excellent alternative to submitting serum for routine NHP serology testing. Microsample eliminates the steps, reagents, material, and equipment needed to prepare and ship serum samples thus saving labor and time in collection and shipping of test samples. In addition, use of a microsampler reduces overall stress in NHPs during collection of blood for routine serosurveillance or from study animals and fits in with the 3Rs animal welfare principle.

Treatment of Skull Osteomyelitis in an Adult Rhesus Macaque (*Macaca mulatta*) with an Acrylic Cranial Implant

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A 10-y-old intact male rhesus macaque (*Macaca mulatta*) presented for significant chronic weight loss and purulent discharge along the margins and within the recording chamber of his acrylic cranial implant. Relevant medical history includes a traumatic fracture and repair of the cranial implant acrylic 2 y prior and a craniotomy 2 mo prior to the current presentation. During the craniotomy, purulent discharge was noted, cultured, and treated with antibiotics. Serial complete blood count and serum biochemistry panels were performed due to continuation of clinical signs and revealed a chronic leukopenia and anemia. Multiple anaerobic and aerobic bacterial cultures and sensitivities were obtained from recording chambers and implant margins, and infections were treated with various antibiotics regimens including metronidazole, trimethoprim sulfa, enrofloxacin, doxycycline, and ceftriaxone. The patient failed to gain weight despite antimicrobial treatment. A computed tomography (CT) scan of the skull revealed osteolysis of the skull under the acrylic implant. The patient underwent a surgical procedure to remove the implant, which was completed in sections using a bone saw and dremmel. Implant removal revealed significant caseous debris, purulent discharge, and granulation tissue over the skull and implant margins. The entire implant was removed due to significant infection and osteolysis over the rostral skull exposing dura. Bacterial culture and sensitivity of the skull surface indicated *Enterococcus* species growth. The tissue was lavaged copiously with vancomycin-polymixin B flush to remove remaining infectious debris. Calcium sulfate beads impregnated with vancomycin were placed around the bone defect before closing the incision. Postoperatively, the patient was administered enrofloxacin and trimethoprim sulfa for 4 wk. There were no noted operative or postoperative complications and the animal gained weight and returned to a body condition score of 3/5 within 6 wk. Bloodwork was repeated 12 wk postoperatively and revealed a white blood cell count and hematocrit within normal reference ranges. A follow-up CT performed 12 wk postoperatively showed bone regrowth, and the defect decreased in size by approximately 50%.

Pseudoaneurysm and Arteriovenous Fistula in a Rhesus Macaque (*Macaca mulatta*)

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An 8-y-old female rhesus macaque (*Macaca mulatta*) presented for a swelling on the left lower limb distal to the inguinal region

directly above the femoral artery. Physical and ultrasound examination revealed a probable combination of an arteriovenous fistula and pseudoaneurysm. After review of possible treatment options described here, it was determined that open surgical repair was the best course of action. The pseudoaneurysm and arteriovenous fistula were surgically resected and the animal recovered without complication.

Cranial Recording Chamber Infection with Multidrug Resistant Bacteria in a Squirrel Monkey (*Saimiri sciureus*)

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A 6-y-old male squirrel monkey (*Saimiri sciureus*) presented with reduced activity 8 d after an IACUC-approved chamber implant surgery. Decreased activity persisted for 2 d despite pain management (meloxicam, 0.1 mg/kg PO). No clinically abnormalities were noted on CBC or blood chemistry. Amoxicillin/clavulanic acid therapy was provided for 10 d. Under isoflurane gas anesthesia, the external portions of the head cap device were cleaned with dilute chlorhexidine diacetate solution (0.05%), and the head cap recording chamber was opened under aseptic conditions. A sample of fluid from the head cap recording chamber was collected for culture and sensitivity. The chamber was flushed with dilute betadine and sterile saline and ophthalmic triple antibiotic ointment was applied prior to closing the chamber with a sterilized cap. Activity and behavior returned to and remained normal following the first head cap cleaning. Results from the fluid sample revealed multidrug resistant *Pseudomonas* spp. A second fluid culture and sensitivity obtained 11 d after the first culture revealed many nonhemolytic *Streptococcus* spp but no *Pseudomonas* spp. These *Streptococcus* spp were found to be resistant to all drugs tested except for chloramphenicol. Applying the *APV Cranial Implant Care Guidelines for Nonhuman Primates in Biomedical Research*, we formulated a chamber cleaning regimen including daily Monday-Saturday cleaning under isoflurane sedation using 1.5% hydrogen peroxide mixed with 1% betadine solution in saline rinsed with sterile saline. For 2 wk, a compounded chloramphenicol (1%) ophthalmic ointment was instilled inside of the cleaned and dried recording chamber after cleaning. After 3 wk, the chamber was confirmed free of *Streptococcus* spp, and cleaning was reduced to 3 times per week. Consecutive negative cultures resulted in reduction to twice weekly head cap cleanings and removal of hydrogen peroxide from the cleaning protocol. After 1 mo of twice weekly head cap cleanings, cytology confirmed the presence of many yeast (but no bacteria) within the recording chamber. Sequential trials of hydrogen peroxide then Dakin's solution failed to eliminate the yeast. After approximately 6 mo of consistent head cap disinfection, the squirrel monkey reached experimental endpoint and was euthanized. Histopathology of meninges revealed evidence of chronic inflammation. This case illustrates the need to tailor therapy to changing environments within a recording chamber and how with meticulous care even a chronically infected chamber can be maintained with no adverse effects to the animal and the science.

Malignant Melanoma in a Male Cynomolgus Macaque

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A 3-y-old male cynomolgus macaque (*Macaca fascicularis*) housed in an indoor/outdoor type housing was presented with a small 2 mm diameter, raised black dermal lesion on the left side of his genitalia (scrotum). Excision biopsy of the mass was sent for cytology and histopatology evaluations. Cytology revealed some large intracytoplasmic dark brown to black material suspected to be melanin. Histology revealed large polygonal dermal cells that were tightly packed in some areas and loosely arranged in others. These cells had large oval nuclei, granular chromatin, small nucleoli, and abundant cytoplasm containing numerous dark brown granules (melanin). No mitotic figures were seen. A blood vessel in the subcutis had tumor cells in its walls. The final diagnosis was malignant melanoma. Radiographs of the animal's thorax and abdomen were taken to assess potential metastasis. A 2-mo follow up examination revealed complete healing of the skin and no recurring melanoma. Malignant melanoma is rarely reported in nonhuman primates and is seen in other animal species including rabbits, dogs, cats, and horses. Etiology in humans may be based on UV ray exposure in light skinned people (but can occur in anyone) and if caught early may be surgically excised with a good prognosis. If caught late, the prognosis is poor with extensive metastasis to lymph nodes, lungs, other organs, and bone. Current treatments also include chemotherapy, radiation therapy, immunotherapy, and targeted therapy.

Longitudinal Anthropometric Assessment in Transgenic Huntington's Disease Monkey (*Macaca mulatta*) model

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The neurodegeneration associated with Huntington's disease (HD) leads to the onset and advancement of motor and cognitive impairment with increased age in humans. In children at risk for HD, body measurement growth abnormalities exhibited include a reduction in body mass index (BMI), weight, height, and head circumference. We developed a transgenic HD monkey model first reported in 2008. This study discusses the progression of body measurements in our first generation HD monkeys from infancy through adulthood. The growth of our HD monkeys was assessed by measuring head circumference, sagittal, and transverse head measurements, crown-to-rump ("height") measurements, and BMI. The animals were evaluated monthly from 0 to 72 mo of age and every 3 mo from 72 mo of age onward. Compared to wild-type control monkeys, HD monkeys displayed increased BMI, head circumference, and sagittal head measurements. The physiological comparability between nonhuman primates and humans underscores the translational utility of our HD monkeys to evaluate growth and development patterns associated with HD.

Elevated D-Dimers in an Adult Pregnant Female Rhesus Macaque (*Macaca mulatta*)

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An 11-y-old Indian-origin, female rhesus macaque (*Macaca mulatta*) presented with severe fight wounds consisting of diffuse crush injuries. Ultrasound examination revealed a late-term nonviable fetus with a biparietal diameter of 43 mm, indicating a 143-d gestational age fetus. A complete blood count, serum chemistry, and urinalysis were consistent with muscle injury and revealed a urinary tract infection with no evidence of secondary renal compromise. Aggressive fluid therapy and broad spectrum antibiotics were initiated. Following stabilization, a coagulation panel revealed decreased prothrombin (PT) and partial thromboplastin time (aPTT). D-Dimers were markedly elevated at 422 ng/mL. This is 3 times higher than published reference ranges and significantly higher than inhouse controls submitted for comparison (age-matched, trauma- matched, and infection-matched). Cesarean section was elected for removal of the nonviable fetus. Necropsy of the fetus and placenta revealed a moderate, fibrinous subacute, multifocal-to-coalescing placentitis that was phosphotungstic acid

haematoxylin (PTAH) stain positive, indicative of fibrin deposition in the placenta in the zone demarcating normal from necrotic tissue. Within this region of distinction, focal areas of fibrin clots consistent with an ischemic event were apparent. At this time it is unknown what distribution of thrombi are necessary to induce fetal death in humans or macaques though an association has been established in human literature. In this case, elevated D-Dimers are indicative of a hypercoagulable state that may have resulted in microthrombi in the placenta and subsequent late term fetal loss. In humans living in developed countries, thromboembolic complications are the leading cause of both maternal and fetal mortality. Pregnant women exhibit a progressive increase in plasma D-Dimer levels throughout the course of gestation. This increase corresponds in magnitude to the risk of developing deep vein thromboses during pregnancy. We are currently in the process of establishing whether a similar increase occurs during pregnancy in rhesus macaques, and whether elevated D-dimer levels are associated with an increased risk of late term fetal loss for individual female macaques in our colony.

Malignant Lymphoma in a Sooty Mangabey (*Cercocebus atys*)

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A 22-y-old female sooty mangabey (SM, *Cercocebus atys*) presented for weight loss, lethargy, and intermittent lateral recumbency. The animal was assigned to an AIDS research study and was inoculated with SIVsmm 15 y prior. Multiple blood samples and lymph node biopsies were collected throughout her lifespan. Physical exam revealed a necrotic and ulcerated lesion in the mouth and marked submandibular lymphadenopathy. Thoracic radiographs revealed multiple radiopaque, variably sized nodules throughout right and left lung lobes. Due to poor prognosis, the animal was euthanized, and submitted for a necropsy examination. Pathologic findings revealed a B-cell lymphoma affecting multiple lymph nodes, spleen, tonsils, and lungs. Malignant lymphoma has been described for decades in a variety of nonhuman primates (NHPs). However, many of the original descriptions of lymphoma in these species were made prior to the discovery of oncogenic viruses. In SMs, Simian-T-lymphotropic virus (STLV)-1 has been reported to cause lymphoproliferative disease similar to acute lymphoblastic leukemia in humans. Epstein Barr virus (EBV) is known to cause lymphoma in macaques, but no EBV associated lymphomas cases have been documented in SMs. The animal in this case was tested negative for STLV and EBV. SIV infections are nonpathogenic in their natural simian hosts, including SMs, and multiple studies have reported that SIV-infected SMs remain disease free for up to 24 y despite relatively high levels of viral replication. Rare cases of classic AIDS have been described in naturally or experimentally infected SMs. The lesions in this animal did not reveal presence of giant cells as seen in classic AIDS cases. Therefore, possibility of spontaneous development of B-cell lymphoma is speculated in this case.

Sudden Death in a Cynomolgous Macaque (*Macaca fascicularis*)

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A 3 y-old male cynomolgous macaque (*Macaca fascicularis*) of Cambodian origin presented for necropsy after being found dead in his cage. The animal had been released from internal quarantine 2 d prior, had not yet been assigned to study, and had appeared clinically normal when observed that morning. On gross examination, the animal appeared in good body condition with normal hydration and fat stores. No significant lesions were observed until the calvarium was opened to reveal moderate quantities of yellow-green purulent discharge originating from the cerebellum. Swabs of the material were taken for bacterial culture and sensitivity and a standard tissue set, including the brain, was collected for histologic examination. Histologic examination revealed a focal abscess within the cerebellum that was composed of necrotic debris and inflammatory infiltrates, accompanied by occasional clusters of gram-negative rods. Additionally, there was multifocal, marked expansion of the cerebellar and cerebral meninges with large numbers of neutrophils and lesser numbers of macrophages. Multifocal aggregates of lymphocytes and macrophages surrounded meningeal vessels as well as vessels within the pia mater in sections of the spinal cord. Neutrophils were also noted to infiltrate the pia mater and follow along nerve roots. The remaining organs examined were overall histologically unremarkable. Culture and sensitivity testing conducted in conjunction with PCR revealed a preliminary diagnosis of *Burkholderia pseudomallei*; this was confirmed with immunohistochemical staining of brain and spinal cord and additional PCR testing. While *B. pseudomallei* is designated as a select agent by the CDC due to its pathogenicity and zoonotic potential, it is endemic in Southeast Asia and Australia. Therefore, animals imported from these regions may unintentionally introduce the bacterium into the laboratory environment, putting both other animals and humans at risk, as was demonstrated in this case.

The Combination of Butorphanol, Azaperone, and Medetomidine (BAM) for the Immobilization of Rhesus Macaques (*Macaca mulatta)*

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Maximizing animal welfare by minimizing drug-related side effects is a major consideration when choosing pharmaceutical agents for chemical restraint in nonhuman primates. This is further supported by the principle of refinement which dictates that efforts should be made to reduce animal distress and discomfort during scientific procedures. A novel drug that may promote these ideologies is the combination of butorphanol (2.48 mg/mL), azaperone (0.83 mg/mL), and medetomidine (0.99 mg/mL) (BAM), developed for the immobilization of wildlife as an alternative to ultrapotent opioids. The potential benefits of BAM include anesthesia and analgesia in a single, low-volume injection with agent reversibility. This study compared 2 doses of BAM (16 uL/kg IM, 24 uL/kg IM) in healthy juvenile 3-y-old rhesus macaques (n = 12 male and 12 female). Physiologic parameters including heart rate, rectal temperature, arterial hemoglobin saturation (SpO2), and mean arterial pressure (MAP), as well as anesthetic quality assessments (pedal reflex, spontaneous movement), were recorded every 5 min. Experimental endpoints were established for heart rate (≤ 80 bpm for 2 consecutive readings), MAP (≤ 50 mmHg), hypoxia (≤ 85% SpO2), and hypothermia (≤ 97 F), which if achieved, immobilization was reversed with atipamazole (0.22 mg/kg IM). Induction was rapid and smooth for all animals (4.04 +/- 1.24 min); however, both groups exhibited initial clinically significant hypoxia and developed hypothermia over time. Animals in the low-dose group had higher heart rates and MAP compared to the high-dose group. Although BAM provided immobilization for both dose groups, the majority of animals (83%) reached established experimental endpoints for bradycardia or MAP regardless of dose. Based on the results of this study, the use of BAM for the immobilization of rhesus macaques may have limited utility and did not result in the enhancement of animal welfare.

Apocrine Gland Adenocarcinoma of Dermis and Subcutis Tissue in a Rhesus Macaque (*Macaca mulatta*)

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During routine physical exam, a 14-y-old outdoor-housed, female Rhesus macaque (*Macaca mulatta*) presented with a superficial cutaneous mass in the lower left quadrant of the abdomen and considerable weight loss. Grossly the mass was firm, multilobulated, ulcerated, and measured 3 cm ´ 1.5 cm. The mass was surgically excised, fixed in buffered formalin, and submitted for histopathological evaluation. Hematological results were within normal range. However, hypoalbuminemia, with marked elevation of the hepatic enzymes (ALT, AST, and ALKP) were reported on serum chemistry analysis. Abdominal and thoracic radiographs revealed areas of increased radio density with a diffuse distribution. The animal was euthanized due to continued weight loss and poor prognosis. At necropsy, hepatomegaly and multifocal hepatic raised white nodules were observed. The lungs had similar multifocal, variable sized, white, raised nodules scattered throughout. Histologically, the mass was well demarcated but nonencapsulated and composed of cuboidal cells arranged in a glandular pattern with markedly desmoplastic stroma. The neoplastic cells had round-to-oval nuclei, a centrally located nucleolus, moderate cytoplasm, and distinct cell borders. The final diagnosis was apocrine gland adenocarcinoma. Apocrine gland adenocarcinoma, a subtype of sweat gland carcinoma, are rare with only a few cases found in dogs, cats, and humans. Many of these carcinomas are indolent, slowly developing, locally aggressive, but rarely metastatic. The potential of metastasis is considered in this case, given the findings in the liver and lungs. To the author's knowledge, this is the first report of an apocrine gland adenocarcinoma in a nonhuman primate.

Review of Nonhuman Primate Medical Records from 25 CDC Quarantine Groups for Weight Change, Diarrhea, and Skin Disease from 2011—2015

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Data from 25 separate CDC quarantine groups from the years 2011 through 2015 were reviewed in support of an exemption request to the IACUC. The underlying hypothesis was the program of care during quarantine produced healthy animals as noted by few animals losing weight, that minimal significant disease occurred, and the husbandry practices caused no harm. Three criteria were identified as indicators of animal health, including body weight, presence of skin disease, and diarrhea. The medical records from 2,260 animals were examined. The results of the data collected demonstrate 1,213 (53.7%) animals with increased body weight, 919 (40.6%) with stable weight, and 128 (5.7%) with decreased body weight. The prevalence of skin disease was 0 (0%) animals and the incidence 2 (0.1%) animals. The prevalence of diarrhea was 2 (0.1%) animals and the incidence 6 (0.3%) animals. All 25 groups cleared quarantine. We believe the results support the hypothesis based on the results; that 94% of all animals either gained or had a stable body weight, <0.4% of animals had either skin disease or diarrhea. Therefore the current standard of care does not cause harm and that adapted animals are released from quarantine.

A Standard Growth Cut-Off for Diarrhea-Associated Growth Stunting in Outdoor-Housed Infant Rhesus Macaques (*Macaca mulatta*)

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Repeated insults from enteric pathogens may lead to chronic inflammation of the gastrointestinal tract resulting in growth stunting, malnutrition, cognitive impairment, and a condition called environmental enteropathy/enteric dysfunction (EE/EED). Growth stunting from EE/EED is an international health issue, and the World Health Organization has published growth charts to monitor stunting. Only recently, EE/EED-like diarrhea-associated growth stunting was been reported in rhesus macaques at the ONPRC, but infant rhesus macaque growth standards have not been established to monitor for the condition. We hypothesized body-weight growth following the 2 standard deviations below predicted mean of healthy infants would act as a cut-off criteria for screening infant rhesus macaques for diarrhea-associated growth stunting. We used healthy outdoor-housed infant rhesus macaque weight records from 2010 through 2014 to develop a multiple linear model of predicted body weight-for-age with coefficients for housing type and sex. A second set of all outdoor-housed infant rhesus macaque weight records from 2015 through 2016 were split into 4 quartiles by age 0-90 d, 91-180 d, 181-270 d, and 271-365 d and compared to the cut-off criteria of 2 standard deviations below the predicted mean from the body weight model. Weight records in quartiles corresponding to infants aged 91-365 d contained a significantly greater proportion of diarrhea weight records than healthy weight records. Additionally, the negative and positive predictive values cut-offs for infants housed in medium-size shelter breeding groups outperformed cut-offs for infants housed in large-size corral breeding groups. The infants falling below 2 standard deviations of standard growth could be identified at semi-annual physical exams and assessed for diarrhea-associated growth stunting and EE/EED.

Adventures in Model Development: Rhesus Macaques, Diarrhea, and Environmental Enteric Disease

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Enteric disease results in approximately 2 million deaths annually, including 760,000 children under the age of 5. Repeated or unresolved enteric infections may damage the intestinal tract, resulting in a condition commonly referred to as environmental enteropathy (EE) or environmental enteric disease (EED). This condition of poor nutrient absorption is broadly defined by the observation of histologic abnormalities in the small intestine in which villus architecture changes from the normally long finger-like villi to shorter, blunted and fused villi with decreased surface area for efficient nutrient uptake. Socially housed infant rhesus macaques are highly exploratory, and engage in behaviors associated with fecal/oral transmission of gastrointestinal pathogens. We hypothesized that these infants naturally develop a spectrum of symptoms, from inapparent environmental enteric disease to chronic enteric inflammation similar to human EED. A retrospective analysis of a large-scale cohort of infant rhesus confirmed an association between diarrhea and growth stunting in outdoor-housed animals at ONPRC, and retrospective analysis at both ONPRC and CNPRC demonstrated that outdoor-housed infants have histologic changes in the small intestine similar to human EED patients. From this pilot data, we initiated a multicenter longitudinal cohort study, following 80 animals from 1 to 8 mo of age. We evaluated anthropomorphic measures, rectal fecal cultures, histologic changes, and xTAG PCR microbiome analysis. The enteric pathogen burden differed between the 2 centers, subclinical intestinal inflammation was widely distributed, and growth stunting correlated with severity of inflammation and enteric pathogen burden. Socially housed rhesus macaque infants appear to closely model human EED. Screening therapeutic interventions may enhance human drug development and contribute to improved health of rhesus macaque breeding colonies.

Feasibility of Open Heart Surgery for Mitral Valve Replacement in the Baboon Model (*Papio hamadryas*)

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Critical congenital valvular heart disease in newborn infants remains one of the most difficult and challenging problems to treat. Prosthetic valves are unavailable in small sizes, nor can they grow with the child. Where feasible, patients will require multiple operations over the course of their childhood as they grow. One area that may hold promise is the use of extracellular matrix scaffolds for the repair of congenital heart defects. Porcine extracellular matrix is a material currently available and is approved by the FDA for intracardiac and extracardiac use, which has shown to promote cardiovascular tissue deposition and remodeling. Moreover, it may also support somatic growth. Different animal models have been used to perform mitral valve replacement, particularly ovine and porcine models. However, particularly for valves, these models do not mimic the human response. Pannus overgrowth and thrombus complications are common in ovine and porcine models, respectively. On the other hand, the baboon model (*Papio hamadryas*) is anatomically and physiologically closer to human and may be ideal for scaffold valve implantation in the mitral position during cardiopulmonary bypass and subsequent evaluation. Surgical approaches involved a right thoracotomy, with aortic and bicaval cannulation, during arrest of the heart accomplished with a single dose of custodiolcardioplegia. Mitral valve access was via Sondergards groove and a left atriotomy. Initial hemodynamic of the valve showed outstanding results with no signs of stenosis or obstruction, and minimal, if any regurgitation. While these early results are clearly encouraging, the valve was only tested in the short term. Further studies with long-term follow up will provide additional information on scaffold valve growth potential.

Seroprevalence of West Nile Virus in an Outdoor Nonhuman Primate Breeding Colony Located in South Florida

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West Nile Virus (WNV) was first reported in New York State in 1999, becoming endemic in the United States over the next few years. In the state of Florida, WNV was first identified in 2001, and 360 human cases have been reported as of January 2017. This is likely due to the consistently warm, humid, and rainy Florida climate—ideal for mosquitoes (*Culex* spp.) which transmit the disease. Two previous large-scale serologic surveys screened outdoor nonhuman primate colonies at the Tulane National Primate Research Center (TNPRC) and Yerkes National Primate Research Center (YNPRC), resulting in marked differences in overall seroprevalence (TNPRC 36%, YNPRC 3%). We investigated the seroprevalence of 3 outdoor-housed nonhuman primate species at The Mannheimer Foundation, Inc. located in South Florida. A total of 520 subjects, at least 4 y of age, were sampled between our 2 facilities, including 200 rhesus macaques, 212 cynomolgus macaques, and 108 hamadryas baboons. Serum samples were collected from January 2016 to February 2017 and submitted to the University of Georgia Veterinary Diagnostic Laboratory to determine the presence of WNV IgG antibodies via serum neutralization assays (SNA). Additionally, a subset of samples was submitted to Idexx Bioresearch for detection of WNV antibodies by multiplex fluorescent immunoassay 2 (MFIA 2). Confirmatory testing was performed on a small number of samples at the Cornell University Animal Diagnostic Center via plaque reduction neutralization test (PRNT). The results yielded an overall seroprevalence of approximately 20%, with the highest in baboons (37%). Although there were differences in macaques between our 2 sites, the overall seroprevalence was 13% for the rhesus macaques and 17% for the cynomolgus macaques. The seroprevalence of antibodies against WNV detected in these nonhuman primate colonies highlights the importance of vector-borne disease transmission and surveillance in research animals housed in climates where these vectors thrive.

Subcutaneous Melengestrol Acetate (MGA) Implant as a Treatment for Endometriosis in Rhesus Macaques (*Macaca mulatta*)

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A 19-y-old, 10 kg female rhesus macaque (*Macaca mulatta*) used in cognitive neuroscience research sedated for routine suture removal was bright and alert, and without clinical signs of illness. On physical examination, a large, firm, palpable mass was present in the caudal abdomen. Abdominal radiographs revealed a large soft tissue-opaque mass in the caudal abdomen, associated with the uterus. The diagnosis of endometriosis was confirmed by visualizing a 12 cm ´ 16 cm ´ 10 cm, hypoechoic, fluid-filled cyst on ultrasound examination, and aspiration of 140mL of chocolate-colored fluid. Endometriosis in rhesus macaques is commonly treated by once monthly IM methylprednisolone acetate injections. However, as this particular animal was no longer needed by the investigator for neuroscience research, she was eligible to be transferred to a sanctuary for retired research primates, so an alternative, long-lasting treatment option was explored. In humans, endometriosis can be successfully treated with the use of progesterone analog contraceptives, including subcutaneous progestin implants. Subcutaneous implants impregnated with 20% MGA have been successfully used in primates and other species in zoos for contraceptive purposes. In this case, the female macaque was treated a MGA implant that was placed subcutaneously between the scapulae. Serum progesterone and estradiol levels were monitored by LC/MS/MS, which is specific for endogenous hormones, at 2 time points pre (48 mo: progesterone 2260 and estradiol 11.6 pg/mL and 1 mo: progesterone 9810 and estradiol 36.6 pg/mL) and posttreatment (2 mo: progesterone 3.24 and estradiol 18.5 pg/mL and 4 mo: progesterone 3.0 and estradiol 16.1 pg/mL). These results, along with regression and involution of the cyst monitored by serial ultrasonography, suggest that the luteal phase of the menstrual cycle is being maintained by the implant. This report demonstrates the utility of a subcutaneous MGA implant for the treatment of endometriosis in a laboratory rhesus macaque.

Large Intestinal Polyps in a Cynomolgus Macaque (*Macaca fascicularis*)

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Colorectal carcinomas are common causes of cancer-related morbidity and mortality in humans in North America. Most begin as benign polypoid adenomas. Polyps are discrete masses of epithelial tissue that protrude into the large intestinal lumen. Although common in humans, pedunculated polyps have not been described in cynomolgus macaques (*Macaca fascicularis*), and only 10 cases have been reported in rhesus macaques (*Macaca mulatta*). Benign adenomatous polyps are the progenitors of most colon cancers, with larger size, villous histology, and higher degree of dysplasia being the factors that correlate best with malignancy. Neoplastic transformation has 2 phases, tumor initiation and tumor development. Tumor initiation involves the formation of the adenoma, and is often attributed to the loss of the tumor suppressor APC (adenomatous polyposis coli) gene function. Tumor progression from adenoma

to carcinoma occurs due to tumor-promoting factors. Aside from a protracted history of intermittent diarrhea, a 9-y-old, female cynomolgus macaque had no other physical or clinical abnormalities. Several years before necropsy, 2 samples of tissue were collected from the catch pan of the animal's cage and submitted for histologic evaluation. Neither of the samples was well preserved, making interpretation equivocal. The first was suspected to represent a spindle cell tumor, and the second had abundant mucus, possibly representing a mucus-producing adenocarcinoma. Necropsy was performed at the animal's experimental endpoint after euthanasia. Nine polypoid mass lesions ranging from 0.25 ´ 0.25 ´ 0.25 cm to 2 ´ 1.5 ´ 0.5 cm were identified in the cecum and colon. Additionally, about a dozen smaller red foci slightly raised the colonic mucosa, and the mesenteric lymph nodes were prominent. Microscopically, the polyps were supported by dense cores of fibrous connective tissue, covered by well differentiated enterocytes with abundant goblet cells, and had hemorrhages in the superficial lamina propria. Enterocyte mitoses occurred at 2-12 per 40 ´ field. Paneth cells were occasionally present among the deeper glands. Some of the polyps were ulcerated and inflamed. The glands were often dilated and filled with mucin, and plump fibroblasts were present along the margins. In some areas, the glands infiltrated the fibrous stalk. Staining with Masson's trichrome demonstrated abnormal thinning, thickening, and in some areas, complete absence of the muscularis mucosa, as well as variably sized accumulations of disorganized smooth muscle infiltrating the fibrous cores. The final diagnosis was large intestinal polyps with atypia.

Does Chronic Social Stress Impair the Prenatal Transfer of Anti-Tetanus Immunity in Captive Breeding Female Rhesus Macaques (*Macaca mulatta*)?

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Tetanus can cause significant morbidity and mortality in nonhuman primates (NHP); therefore, colonywide vaccination with tetanus toxoid is recommended for outdoor breeding colonies of rhesus macaques with primary immunizations commonly given to infants at 6 mo of age followed by booster vaccines every 10 y. Maternal antibodies are thought to offer protective immunity to infants ≤ 6 mo of age. However, historical colony data from our institution show a higher incidence of tetanus among infants (≤ 6 mo old) born to subordinate dams. It is unknown whether this higher incidence of infantile tetanus is due to a higher incidence of trauma among subordinate animals or a stress-induced impairment of maternal antibody protection. Studies in other NHP species suggest that chronic exposure to social stressors interferes with the transplacental transfer of IgG via its receptor. Thus, the primary aim of this study was to determine whether chronic stress associated with social subordination impairs the prenatal transfer of anti-tetanus immunity in breeding female rhesus macaques. Subjects included 26 high- and 26 low-ranking adult female rhesus macaques either near 5 or 10 y postinitial immunization (pi) and their nonimmunized infants. We hypothesized that infants born to subordinate dams near 10-y pi would have the lowest infant to dam antibody ratios and thus would be at greatest risk for infection. Results revealed no significant difference among the vaccine groups in infant antitetanus IgG levels. However, infant-to-dam antibody ratios were lower among subordinate animals compared to more dominant animals, after covarying for number of ys since dam's initial vaccination (P <0.05). Additionally, higher hair cortisol levels predicted lower infant to dam antibody ratios (P <0.05). Together, these findings suggest that chronic social stress in female rhesus macaques may impact the prenatal transfer of antitetanus immunity to the offspring.

Liver Mass in a Tufted Capuchin (*Cebus apella)*

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A 36-y-old female tufted capuchin (*Cebus apella*) presented acutely moribund. The animal was euthanized, blood was collected, and the body frozen for later necropsy. On postmortem examination, the animal was thin, the left ventricle of the heart appeared thickened, and there was a large, solid, hemorrhagic mass (220g) affecting the right lobes of the liver. The gallbladder could not be expressed, indicating cholestasis. The only other gross findings were cystlike structures replacing the normal architecture of the left kidney. Based on the appearance and location of the mass, hemangiosarcoma was suspected with differential diagnoses of hematoma, hemangioma, hamartoma, telangiectasia, and hepatocellular carcinoma. The complete blood count showed elevation in many liver enzymes and products indicating cholestasis (GGT, total bilirubin) with some hepatocellular damage (ALT, AST). Alkaline phosphatase was within normal limits. Hepatocellular function was impaired based on elevated total bilirubin and BUN, but decreased total albumin, which could be attributed to decreased liver function or hemorrhage. Cholesterol was normal but hematocrit was very low (7.28%), making hemorrhage a more likely cause of hypoproteinemia. Red cell volume and hemoglobin were within normal limits, indicating no impairment of iron metabolism. These findings are consistent with the gross pathology related to a hemorrhagic, space-occupying mass replacing functional liver tissue and compressing the common bile duct. Histopathology revealed no signs of malignancy, which made hemangiosarcoma unlikely. Therefore, the presumptive diagnosis is hemangioma with further differentials being hamartoma and telangiectasia. To our knowledge, this lesion has been described rarely in NHP and never in *Cebus apella*. The renal findings are common in elderly individuals of this species and likely incidental. The right kidney appeared to be normal and creatinine was nearly normal (2.1), so BUN was likely attributable to the liver lesion. Overall, this case demonstrates a previously undescribed lesion in *Cebus apella*.

The Ongoing Search for Improved Diagnostic Tools to Detect Tuberculosis in Nonhuman Primates

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One of the major concerns in nonhuman primate colonies continues to be routine screening of animals for tuberculosis. As the standard method is the tuberculin skin test (TST), which can be prone to false positive results due to technique, cross reaction with other *Mycobacteria* sp., or other limitations, we and others continue to search for additional TB testing tools to use in screening colonies. A commercial vendor is continuing to explore the use of antibody testing as a marker. Our data has not identified a single satisfactory antigen target, but suggests that a panel will be necessary for accurate testing, making the multiplex microbead assay a suitable platform. We are currently refining a less-than-10-member panel that yields greater than 95% specificity with samples from uninfected nonhuman primates (including a subset with confirmed *M. avium* reactivity). We have observed a variable range of sensitivities using samples from naturally or experimentally exposed or infected nonhuman primates from various studies. Since the commercial assay interferon gamma stimulation assay is no longer available, the California National Primate Research Center Pathogen Detection Laboratory has restarted our previous studies to evaluate various stimulation antigens and cytokine release levels are a marker for cell mediated immunity. Similarly to what we have observed with

antibody, the cytokine release data generated thus far indicates good specificity but a variable range of sensitivities. Given the specificity data observed thus far, positive reactivity with either of these assays appears to be useful in confirming a positive skin test. However, the variable reactivity we are observing with positive samples from different studies makes it difficult to effectively evaluate and validate the sensitivity of these assays. Further development of assays would benefit significantly from testing at sites that encounter spontaneous cases of TB that can be confirmed through other methods.

A New Host of Simian Retrovirus (SRV)

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Exogenous type D retrovirus found in nonhuman primates includes SRV-1 through -8. The natural hosts of SRV are macaques (*Macaca mulatta*), Hanuman langurs (*Semnopithecus entellus*), Ethiopian baboons (*Theropithecus gelada)*, and African green monkeys (*Chlorocebus*). Here we identify a new SRV carrier, mandrill (*Mandrillus sphinx*), which has never been reported as one of the hosts of SRV before. In 2013, a rhesus macaque (#A) received a Plasmodium inoculation from cryopreserved blood of a rhesus monkey (#B). Rhesus #A was thereafter seroconverted from antiSRV antibody negative to positive during annual screening. SRV was also detected by PCR and virus was isolated from the whole blood of rhesus #A. We hypothesized that the source of this SRV infection was from the cryopreserved blood of mandrill (collected in Gabon in 1986) that was given to rhesus #B (in 1991) and then transmitted to rhesus #A. Testing mandrill cryopreserved blood showed that SRV PCR was positive, SRV was successfully isolated, but SRV antibody test was negative. Comparison of gag, prt, and partial env gene amplified from mandrill SRV and rhesus SRV demonstrated 100% matched and confirmed that both viruses were identical. Sequence analysis of 3'pol-env gene showed 94% identical to a SRV-2 reference strain (D2/RHD/OR) isolated in the U.S. Phylogenetically, mandrill SRV was more closely related to rhesus SRV-2 than baboon SRV based on gag gene sequences. This is the first report of discovery of a SRV-2 variant strain isolated from a wild mandrill in Africa. Interestingly, mandrill SRV-2 was closely related to rhesus SRV-2 although their geographically origins were on the different continents.