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Abstracts of Scientific Papers

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Oral Case Reports

Necrotizing Dermatitis in a Rhesus Macaque (*Macaca mulatta*)

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A 3-y-old female rhesus macaque (*Macaca mulatta*) presented with minor crush trauma to her upper left thigh, inflicted by a female conspecific. Wounds were treated topically and appeared to be healing three days post injury. Eight days post injury the wound had deteriorated. A necrotic area was noted and cultured 3+ *Staphylococcus* spp., coagulase positive. Topical wound treatment was restarted (hydrotherapy and sulfasalazine), as well as a systemic antibiotic (cephalexin 250 mg BID). Wound continued to deteriorate with spreading areas of necrosis. Animal was sedated daily for wound care and debridement, antibiotic was changed to ceftriaxone (96 mg BID), and analgesic and anti-inflammatory treatment regimens were initiated. Daily treatments included: ceftriaxone (96 mg BID), meloxicam (0.5 mg SID), tramadol (25 mg BID), diphenhydramine (25 mg BID), topical lidocaine to debrided areas, and sulfasalazine topically. Biopsies were obtained from wound margins and diagnosed as necrotizing dermatitis. The wound and associated necrotic tissue continued to worsen for 2 wk, after which point a slow improvement began. Following 5 wk of treatment, the animal was nearly completely healed, and returned to normal activity with her social group. This is the second case of necrotizing dermatitis in the rhesus macaque colony at UT MD Anderson Cancer Center, and it demonstrates that these severe wounds can be resolved with intense care and time, similar to parallel human cases.

Cunninghamella bertholletiae* and *Mycobacterium avium* Coinfection in a Cavitary Lung Lesion of an Immunosupressed *Macaca mulatta

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A research-driven thoracic CT scan of an SIV infected rhesus macaque revealed a cavitary lesion in the lung perenchyma suspicious for *Mycobacterium tuberculosis*. Workup included intradermal OMT testing, gastric aspirate for culture, primagam, and ultimately necropsy with culture and histopath. Results from multiple labs confirm presence of both *M. avium* and *Cunninghamella bertholletiae* in the lesion. Retrospective review of baseline CT scans revealed that a small lesion was present prior to immunosupressiont.

Lymphosarcoma Impairs Results of In Vitro Blood-Based Tuberculosis Assay

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According to EU (European Union) legislation nonhuman primates used for research purposes have to be monitored once a year for infection with *Mycobacterium tuberculosis* (Tb). In the case of Tb positive testing results, the veterinary authority can suspend or even withdraw the license for a primate colony. Up to now we have used Tuberculin skin test (TST) routinely. However, the reliability of this test has recently been questioned because of false positive or anergic response in infected animals. Therefore, in 2011 we tested the entire primate colony with an in vitro blood- (interferon gamma, IFN-γ) based assay with satisfactory results. To confirm last year's results, the entire colony was retested in 2012. We had never seen a Tb infection in our colony in the past 20 y. Here we report about the TB testing results of an adult female African green monkey which had been introduced to our colony 13 y ago and has always been group or pair-housed. At the time of blood sampling for TB testing, a swelling (size of a ping pong ball) at the right breast was detected and removed the next day. The encapsulated tumor was examined and erroneously found to be a breast tumor. The blood samples were shipped to the Prionics Company (Switzerland) for testing and evaluation. Several weeks later we received the results. Only the treated animal of the entire colony tested positive for tuberculosis. A confirmation testing was scheduled and had to also involve the room mates in different cages. Unfortunately, the animal was found moribund in the cage and euthanized. At necropsy, generalized tumor invasion of almost all organs was detected. Eight weeks ago, at the time of blood sampling, only one tumor was detectable. Specific staining methods, histologic examination, and bacterial culture were used to detect the Tb infection in tissues and organs. We found no evidence for a tuberculosis infection. After accurate histologic examination, the tumors were identified as lymphosarcoma. We were able to observe in this case that lymphosarcoma activity can interfere or even generate false positive responses in an IFN-γ-based Tb assay. We would therefore recommend a careful clinical examination of the animals at the time of blood sampling for the blood-based assay.

Plasma Antibody Profiles in Nonhuman Primate Tuberculosis

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Tuberculosis (TB) in nonhuman primates is highly contagious and often produces rapid disease. Identification of animals infected with *Mycobacterium tuberculosis* (M. tb.) in a timely manner is therefore critical. Animals in breeding colonies are periodically tested using the tuberculin skin test (TST) and/or the in vitro blood-based assay blood assay. However, these tests lack desirable sensitivity, specificity, efficiency and throughput. We aimed to develop a blood based immunoassay by exploiting the host immune response against M. tb. Infected animals contain plasma antibodies against M. tb. antigens that can be used potentially for routine colony surveillance. Choice of antigen, however, is difficult because antibody responses against a given antigen are not detectable in all infected animals. We used a panel of 28 antigens in a multiplex immunoassay format where individually identifiable microbead sets were coated with each antigen and used in the simultaneous detection of antibodies against all antigens in a single sample (plasma or serum). Computer assisted multivariate analysis of the experimentally infected (M. tb. strains: Erdman and H37RV) and 135 uninfected animals

of 2 species (61 rhesus and 74 cynomolgus macaques) revealed diagnostically valuable antibody profiles against 8 antigens. All experimentally infected animals contained antibodies against at least one of these antigens. Importantly, plasma antibody profiles in rhesus macaques involved in a TB outbreak ($n = 15$) were studied in naturally acquired M. tb. infection and disease. Animals with lung pathology consistent with TB ($n = 10$), contained antibodies to several additional antigens in a profile similar to that previously reported by us in human TB patients. The above results suggest that the multiplex panel of antigens reported here could be developed as a blood based test for screening of M. tb. in nonhuman primates.

Blastomycosis: A Reemerging Nonhuman Primate Disease?

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During evaluation for chronic normocytic, normochromic, non-regenerative anemia, a 7-y-old female Indian-origin *Macaca mulatta* from the Tulane National Primate Research Center (TNPRC) breeding colony developed hemoptysis. Chest radiographs revealed a diffuse nodular interstitial pattern with cavitary lesions. Anemia was accompanied by an inflammatory leukogram. Cytologic changes on a sample prepared from bronchoalveolar lavage (BAL) included neutrophilic and histiocytic inflammation, with no evidence of any specific causative agent. Severe deterioration of the animal's clinical condition resulted in humane euthanasia and necropsy. Gross necropsy findings included severe multifocal to coalescing pyogranulomatous bronchopneumonia with hemorrhage, and varied amounts of free as well as clotted blood within the oral cavity, esophagus and stomach. Histopathologic examination revealed abundant fungal organisms morphologically compatible with Blastomyces sp. within pyogranulomatous inflammatory lesions in the lungs. Culture confirmed Blastomyces dermatitidis. Retrospective review of animal records revealed additional diagnoses since 1992 in one *Macaca mulatta* and one *Macaca nemestrina* and an additional two *Macaca mulatta* cases recently diagnosed in the last year. In these cases, system involvement varied, but commonly included the pulmonary tract. One case related to experimental simian immunodeficiency viral infection was limited to renal involvement, which appears to be a rare presentation. In Louisiana, Blastomycosis is considered to be endemic and reportable, with an incidence of 1 to 2 cases per 100,000 people; however, St. Tammany Parish, the location of the TNPRC, has had the third highest incidence rate in the state, with 7 cases per 100,000 people.

Extraintestinal *Campylobacter* Infections in Rhesus Macaques (*Macaca mulatta*)

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Campylobacter organisms often colonize the intestinal tract of mammals, birds, reptiles, and shellfish. Many animals infected with *Campylobacter* become asymptomatic carriers. If disease does develop, it is most commonly gastroenteritis with diarrhea. Two cases of clinical disease associated with extra-intestinal *Campylobacter* infection were recently encountered in rhesus macaques (*Macaca mulatta*) at Yerkes National Primate Research Center. The first case, a 3-y-old, male rhesus macaque assigned to an AIDS study, was inoculated with SIV in December 2011 and maintained consistently high viral loads following infection. In May 2012, he presented with suspected abdominal pain and a midabdominal mass was identified on examination. Diagnostics were performed, but due to severity of signs and poor prognosis, euthanasia was elected. At necropsy, a mass filled with purulent material (abscess) was found within the median liver lobe. Fibrinopurulent peritonitis and intestinal serositis were also present. *Campylobacter fetus* was isolated from the blood, liver, and the hepatic abscess. The final diagnoses were *C. fetus* bacteremia and severe multifocal necrosuppurative cholangiohepatitis with intralesional *C. fetus* and *Cryptosporidium* spp. The second case was a 1-mo-old, female rhesus macaque that presented to necropsy in June 2012. The infant was thin and had diarrhea staining present on the perineum. No additional gross lesions were noted. *Campylobacter coli* was isolated from the liver and colon. The final diagnosis was moderate to severe multifocal neutrophilic to lymphocytic hepatitis with intralesional bacteria. The exact pathogenesis of extra-intestinal spread of *Campylobacter* in these cases is currently unknown. The SIV animal was in an immunocompromised state due to SIV infection and the infant (at 1 mo of age) may not have been as immune competent as an adult. These factors may have contributed to their susceptibility to the systemic spread of the disease.

Clinical Use of Sustained-Release Buprenorphine in Rhesus Macaques

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Buprenorphine is widely used as a part of multimodal postoperative analgesia regimens in many species, including nonhuman primates (NHPs). Sustained-release Buprenorphine (SR-Bup) is a recently available formulation which may provide up to 5 d of analgesia with a single subcutaneous dose. This regimen offers a significant improvement in postoperative care, as buprenorphine HCl has traditionally been administered by intramuscular injection every 4 to 12 h to provide adequate analgesia. However, there is no clinical data regarding optimal dosing of SR-Bup for NHPs. We administered SR-Bup to rhesus macaques (*Macaca mulatta*) undergoing vasectomies (expected to experience mild to moderate pain, $n = 3$) as well as animals undergoing a variety of related neurosurgical procedures (expected to cause moderate pain, $n = 4$). We used a range of doses of SR-Bup from 0.12 to 0.2 mg/kg as part of a multi-modal analgesia plan. Animals were observed frequently for signs of postoperative pain or discomfort as well as sedation and reduced appetite (2 potentially significant side effects of opioid treatment). The initial animal undergoing a neurosurgical procedure received 0.2 mg/kg. This dose was associated with significant sedation, with concurrent reduced food intake over the first 12 postoperative hours. The next 3 animals undergoing neurosurgical procedures received 0.15 to 0.16 mg/kg, which appeared to provide adequate analgesia throughout the postoperative recovery period without significant sedation or reduction in appetite. Animals undergoing vasectomies received 0.12 mg/kg. None of these 4 animals showed signs of pain or discomfort after the surgical procedure. None of the animals in this study required additional doses of buprenorphine. We did observe that increased levels of isoflurane were required to achieve surgical anesthesia compared with previous neurosurgical procedures. Review of the medical records for similar neurosurgical procedures using buprenorphine HCl ($n = 11$) revealed that the average concentration of isoflurane used throughout surgery was significant higher with SR-Bup ($n = 4$) (1.68% versus 1.23%, $P < 0.0001$).

Effect of Prolonged Isoflurane, Propofol, and Ketamine Anesthesia on Physiologic Parameters of Neonatal Rhesus Macaques (*Macaca mulatta*)

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Neonatal rhesus macaques are used as animal models in a variety of research programs including those of infectious disease and neurobehavioral development. Facilities maintaining large breeding macaque colonies also face the clinical challenge of working with infant macaques. Both research and clinical situations arise that require anesthesia of these animals. Despite regular use in the biomedical research setting, little normative data exists for very young macaques, especially regarding response to various general anesthetics. The volatile anesthetic isoflurane as well as intravenous propofol and ketamine are commonly used in both humans and nonhuman primates. With the virtual absence of comparative data, we analyzed complete sets of procedural data from neonatal rhesus macaques (day 5 to 7) that were exposed to either isoflurane (n = 5), propofol (n = 4) or ketamine (n = 4) anesthesia for 5 h. All animals were endotracheally intubated and intravenous catheters were placed to facilitate administration of intra-operative fluids, glucose, and vasoactive medications to maintain presumed age specific homeostasis, modeled according to normative values for human neonatal medicine. Animals were mechanically ventilated to counteract respiratory muscle fatigue due to long duration of anesthesia and equipment dead space. Multiple modalities of temperature support were also utilized to maintain normothermia in the patients. During anesthesia the following parameters were recorded every 15 min: heart rate, oxygen saturation, respiratory rate, end-tidal CO_2, blood pressure, and temperature. Venous blood samples were also collected at baseline (time 0), at three time points during anesthesia (time 0.5, 2.5, and 4.5 h) and at 3 h after recovery (time 8 h). Parameters measured at each time point included sodium, potassium, chloride, total CO_2, blood urea nitrogen, glucose, hematocrit, pH, pCO_2, bicarbonate, base excess, anion gap, hemoglobin, pO_2, oxygen saturation and lactate. Other parameters measured included time to intubation, time to extubation, and total intravenous fluids administered during the 5 h anesthetic. Isoflurane caused significantly more hypotensive events (mean arterial blood pressure <40) and required larger total volumes of intravenous fluids to support blood pressure over the course of the experiment compared to ketamine and propofol. Isoflurane also resulted in significantly shorter average time to extubation compared to the other anesthetics. Other physiologic and hematologic parameters were rather well maintained between anesthetic modalities secondary to the above described management. Veterinarians and researchers should carefully consider benefits and challenges of each anesthetic agent in the context of the clinical scenario or the research question under investigation.

A Randomized, Double-Blind, Placebo-Controlled Study to Assess Safety and Efficacy of Lysozyme in Juvenile Rhesus Macaques (*Macaca mulatta*) with Diarrhea

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Lysozyme is a glycoprotein that is produced in high levels in human breast milk and modulates inflammatory responses through nonspecific antimicrobial mechanisms. Infectious and noninfectious insults to the gastrointestinal tract can result in inappropriate inflammatory responses, and lysozyme has the potential to resolve gastrointestinal inflammatory responses. Transgenic goats have been engineered to produce human lysozyme in their milk (hLZ-goat milk), and hLZ-goat milk has been found to help young pigs recover from experimentally induced *E. coli* diarrhea. As diarrhea is a significant cause for hospitalization in young monkeys, we implemented a randomized, double-blind, placebo-controlled study to assess safety and efficacy of hLZ-goat milk in captive, juvenile rhesus macaques (*Macaca mulatta*) presenting to the hospital at the California National Primate Research Center (CNPRC) with diarrhea. Monkeys received initial emergency veterinary treatment and then were assigned to one of 3 treatment groups (n = 5): experimental group (hLZ-goat milk), placebo group (goat milk), and standard-of-care group (oral rehydration fluid). Animals received 5 consecutive days of enteric treatment followed by 5 d of health monitoring. Clinical subjective and objective monitoring parameters were assessed at time of presentation and compared to day of discharge. Hospital diarrhea readmission rates were monitored for 30 d postintervention in order to best assess treatment efficacy. Experimental treatments were well tolerated by all monkeys, and no significant side effects were reported. Enteric administration of hLZ-goat milk resulted in a ≥40% decrease in predicted diarrhea reoccurrence and significantly decreased 1 month readmission rates. Animals administered hLZ-goat milk tended to have improved body condition scores (P = 0.08), and select subjective and objective clinical monitoring parameters were found to return to baseline faster in the hLZ-goat milk treatment group as compared to other groups. As such, lysozyme, as found in hLZ-goat milk, can be considered a safe and effective treatment option for captive, juvenile rhesus macaques with diarrhea.

The Use of Gabapentin in a Juvenile Chimpanzee Following Arm Amputation

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A 3.5-y-old female chimpanzee, native born to University of Louisiana at Lafayette, sustained a severe injury to the left arm while socially housed. Injuries consisted of partial avulsion of the triceps with severing of the brachial artery and avulsion the 4th and 5th digits of the hand. Initially surgical debridement and partial repair of the injury was attempted, however she remained severely depressed for 48 h and necrosis of the remaining fingers of the right hand and the forearm was evident, requiring the amputation of the arm at the scapulohumeral joint. Gabapentin at 10 mg/kg PO Q12 h was added to pain medication and antibiotics after amputation. Three days after surgery the animal began to sit up on her own and groom herself. Four days after surgery she began to climb. Two issues of concern were considered in this case both pertaining to the animal's quality of life: 1) the animal's young age and her ability to adapt to social housing and 2) would phantom limb pain occur? Phantom limb pain occurs in 50% to 80% of humans with an amputated limb and may last for extended periods after amputation. In domestic animals the phenomenon is also presumed to occur. Gabapentin has been implemented as a therapeutic agent to combat the problem in humans and domesticated animals for at least a decade. Gabapentin is effective in several syndromes of neuropathic pain and has recently become widely recommended for use in small animal practice for animals requiring amputation of a limb (VIN). For this animal, Gabapentin was continued for 4 wk in an effort to combat any potential phantom limb pain, she has fully recovered returned to a peer group without incident.

Severe Atherosclerosis and Renal Disease in a Hepatitis C Infected

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A 22-y-old male chimpanzee (chronically infected with Hepatitis C) presented with lethargy and a right stifle wound. Acute blood loss was considered minimal but the animal was pale. Blood work revealed severe microcytic, hypochromic, nonregenerative anemia (HCT = 15.2), azotemia, and elevated liver enzymes. Initial treatments included a blood transfusion, fluid therapy, and wound treatment including antibiotics (cefazolin). Epoetin alpha was initiated 24 h later. The chimpanzee was stable, quiet, alert, and responsive for 6 d, but on day 8, he was recumbent. Humane euthanasia was elected. Gross necropsy identified pericardial fluid, a pale and scarred myocardium, a firm, tan, and scarred liver, and small, tan, firm kidneys with tightly adherent capsules. Histopathologic analysis identified severe chronic interstitial nephritis due to obstructive atherosclerosis and its associated chronic hypoxia. The pericardial fluid and edema present in multiple tissues suggested hypoalbuminemia. There was also proteinuria, and the nephrotic syndrome was suspected. The fourth facet of nephrotic syndrome is hypercholesterolemia; this is interesting in light of the positive feedback loop it could establish with atherosclerosis. The anemia is characteristic of blood loss or iron deficiency, but no site of such loss was histologically identified. Obstructive atherosclerosis was also identified in the heart, leading to fibrosing cardiomyopathy, and in the pancreas. Atherosclerosis is suspected in the carotid artery, as there was also a large infarct in the caudal midbrain. Humans with atherosclerosis commonly have atherotic plaques within the carotid arteries. The chimpanzee had aspirated small quantities of gastric material several days prior to death, as foci of pyogranulomatous inflammation were centered on refractile plant material. The bone marrow showed both myeloid hyperplasia and a dearth of late-stage erythroid precursors (despite epoetin alpha treatment). The myeloid hyperplasia was attributed to aspiration pneumonia. The liver showed characteristic lesions of chronic Hepatitis C infection.

Clinical *Trypanosoma cruzi* Disease after Transplantation in a Cynomolgus Macaque

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A 5-y-old male cynomolgus macaque born and raised within the United States received a heterotopic cardiac allograft as part of a transplant study. Postoperatively, the animal received monoclonal antibodies targeted to specific immune cell lines (anti-CD154, anti-CD28), but no traditional immunosuppressive therapy (neither corticosteroids nor calcineurin inhibitors). At postoperative date (POD) 35 clinical anemia was detected. By POD47 the anemia had worsened (Hb = 2.3g/dL, Hct = 7.3%) and type-matched whole blood transfusions were initiated. After a total of 4 blood transfusions parameters improved to Hb = 5.9g/dL, Hct = 18.7%. Cardiac allograft function remained stable throughout this time. On POD50, Trypomastigotes were identified on peripheral blood smear. Qualitative rtPCR of whole blood identified the organism as *Trypanosoma cruzi*. While initially stable clinically, the animal soon developed sufficient weight loss to necessitate euthanasia on POD64. Necropsy revealed multiple firm nodular lung lesions approximately 1 cm in diameter with circumferential erythema, as well as 0.5 cm to 1 cm diameter off-white, firm material found free-floating within the allograft ventricles. A complete set of tissues was collected for histopathology (results pending). The heart donor and all four blood donor animals tested negative for *T. cruzi* by rtPCR, indicating that disease was likely recrudescence in the face of transplant and monoclonal antibody therapy. *T. cruzi* causes Chagas disease in humans, primarily in Central and South America. Acute symptoms include swelling, lymphadenopathy, fever, and/or myalgia. Chronic clinical signs include anemia, myocarditis, cardiac arrhythmia, and digestive problems and can present up to 20 y after initial infection. Transmission usually occurs via the bite of triatomine bugs, but can also occur from blood transfusion or organ transplantation. Several reports of NHP species with asymptomatic (but serologic or PCR-positive) *T. cruzi* exist in the NHP literature. There are very few cases in the literature involving clinical Chagas disease in NHPs, including sudden death in a chimpanzee (*P. troglodytes*) and encephalitis in a Celebes macaque (*M. nigra*). What makes the presented case unique is the presence of clinical disease (severe anemia) even without the use of traditional immunosuppression. To our knowledge there are no other documented cases of clinical Chagas disease in the transplant literature. Given the outcome of this case it would appear to be advantageous to test all incoming NHP for *T. cruzi*, especially those to be used for transplant or immunomodulatory studies.

Assessment of Risk for Mosquito-Vectorless Transmission of Dengue Virus between Nonhuman Primates in a Research Setting

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WRAIR

Dengue is a mosquito vector-transmitted viral disease caused by four virus serotypes, Dengue virus (DENV) DENV-1, 2, 3, and 4, which cause acute, febrile diseases of varying severity affecting more than 100 million people in the tropics and subtropics each year. Although several Dengue vaccines are in clinical trials none are yet licensed for human use. Nonhuman primates (NHPs), especially rhesus macaques, are the animal model of choice for preclinical evaluation of candidate Dengue vaccines. After DENV infection rhesus macaques may develop circulating virus (viremia) for up to 12 d, but without disease. As highly social animals NHPs have additional requirements which must be considered when these animals are used for research, and pair/group housing to enrich animal welfare is desirable whenever possible. Our study is the first to attempt to model the risk for unplanned Dengue transmission between pair or group housed animals from a bite, scratch or other incidental contact involving the exchange of body fluids. An experiment was performed in which 2 rhesus macaques were initially infected with a high-titered, laboratory-propagated challenge virus stock. A small amount of sera collected during the viremic period from each infected animal was then transferred to 2 Dengue naïve "virtual cagemates" by subcutaneous inoculation to simulate incidental contact resulting in the transfer of body fluids. The testing of sequentially collected blood samples for Dengue viremia demonstrated that both contacts became infected, demonstrating that DENV can in fact be efficiently transmitted between animals by incidental blood-blood contact. Therefore, pair/group housing in studies involving DENV challenge may be contraindicated, at least during the viremic period when the animals are potentially infectious.

Effect of Low-Dose Ketamine on Obsessive-Compulsive Behavior in the Nonhuman Primate

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Obsessive-compulsive behaviors (OCB), such as pacing, overgrooming, and self-mutilation are a common occurrence within captive research nonhuman primate (NHP) colonies. These abnormal patterns of behavior pose a risk to physical and psychologic wellbeing of the NHP and have been considered indicative of compromised welfare. Several projects have tried psychotropic pharmaceuticals to see if the undesired behaviors could be con-

trolled. Recent neuroscience clinical reports show that the use of a low-dose of ketamine (0.5 mg/kg) given intravenously over an hour period has marked positive effect on OCB and depression in human research subjects. Our previous project showed that a single dose (5 mg/kg) of intravenous ketamine on an awake NHP will reduce or eliminate OCB for up to a week but we know that the protocol is not a practical one. Not practical because of frequency and risk associated with administering intravenous drugs on and awake NHP. To assess the practicality of the administration of ketamine in awake NHP we tested if a continuous low ketamine infusion (0.5 mg/kg/h for 1 wk) delivered via an osmotic pump will affect abnormal/undesired behavioral patterns. Three baboons (*Papio anubis*) that show marked pacing, redirected aggression, and/or rocking behavior and that have not received ketamine within the last 6 mo were anesthetized with isoflurane and 4 osmotic pumps were implanted surgically. Their behavior, before and after, the surgical implantations was videotaped in half hour sessions and analyzed for signs of the specific behaviors. Incidence of each behavior was quantified and compared at the different time points. In addition, each observer was asked to give an overall subjective score (0-3) of the intensity of the OCB of the monkey on each half-hour video. Reviewers were blinded as to the treatment stage of the research subject. Our results showed a clear and quantifiable decrease of undesired behaviors on all three subjects with a marked regression to preimplantation baseline levels within 5 to 7 d. This regression correlates with the length the pump was able to deliver the drug.

Spontaneous Diabetes in Sooty Mangabeys (*Cercocebus atys*): Clinical Characteristics, Prevalence, and Risk Factors

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Clinical observations in our colony of sooty mangabeys (*Cercocebus atys*) at the Yerkes National Primate Research Center (YNPRC) suggested that type 2 diabetes occurred at high frequency in this population. Furthermore, postmortem studies of diabetic animals revealed dense amyloid deposits in pancreatic islets. To investigate further, we screened our colony (97 males; 99 females) for the disease. Serum levels of glucose, fructosamine, glycated hemoglobin (HbA1c), triglyceride, and cholesterol were evaluated as indicators of disease. In addition, age, sex, prior exposure to medroxyprogesterone acetate (MPA), and SIV status (positive compared with negative) were evaluated as possible risk factors. Overall prevalence of diabetes was 11%; nearly double that reported in other simian species. An additional 7% were categorized as prediabetic. Among the laboratory parameters evaluated, fructosamine and triglyceride levels were the best indicators of diabetes. Surprisingly, cholesterol and HbA1c were not associated with disease. Increasing age was a significant risk factor with the prevalence of diabetes increasing from 0% in infants, juveniles and young adults to 11% in adults and 19% in geriatric mangabeys. Sex, MPA exposure, and SIV status were not related to disease. Forty-nine mangabeys came to necropsy following clinical euthanasia or death from natural causes and underwent histopathologic evaluation for pancreatic insular amyloid. Twenty-two of these were diabetic, all with pancreatic amyloid, and most with more than 75% of islets replaced with amyloid. We conclude that type 2 diabetes is more common in mangabeys than in other species studied. The disease has some unusual pathologic characteristics in mangabeys, including the absence of altered levels of cholesterol and HbA1c, as well as a robust association of pancreatic insular amyloidosis with clinical diabetes. Future research will examine the genetic basis of mangabey diabetes and will evaluate diagnostic tools based on possible serum indicators of amyloid or amyloid-sensitive imaging techniques.

Fatal Human Herpesvirus-1 Encephalitis in a Black-and-White Ruffed Lemur (*Varecia variegata*)

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A 2-y-old female black-and-white ruffed lemur (*Varecia variegata*) used for public outreach presented because of a 2 d history of inappetence and lethargy that had progressed to seizure activity and status epilepticus. A CBC and clinical chemistry panel from this animal indicated only a mild elevation in creatine kinase, which was interpreted to have occurred secondary to seizure activity. Despite intensive medical management with diazepam and phenobarbital, the lemur died within 2 d of the onset of seizures and was submitted for postmortem evaluation. The lemur was in lean body condition and the only gross abnormality noted were mild, multifocal areas of pulmonary consolidation. Microscopic examination of tissues indicated acute, mild alveolitis and acute nonsuppurative encephalitis with marked focal necrosis and accompanying gliosis within the temporal, premotor, and piriform lobes. Occasional neurons had smudgy, irregular amphophilic intranuclear inclusion bodies and marginated chromatin. General herpesvirus primers were used to amplify a 400 bp region of the genome within the terminase large subunit gene (UL15) from frozen brain specimens. Sequencing demonstrated 100% homology with human herpesvirus-1 (HHV-1). The virus was subsequently isolated in Vero cell culture and identity confirmed with HHV-1 specific primers. HHV-1 has been previously suspected as the cause of fatal encephalitis in a ruffed lemur in the US and diagnosed in a disease outbreak in a group of ring-tailed lemurs in Nigeria. These animals were all held in zoos. This is the first report to positively identify HHV-1 as the causative agent of fatal disease in a lemur using PCR. The source of the virus was not identified, but was most likely contracted from an asymptomatic human through uncontrolled contact. This case demonstrates the critical importance of implementing appropriate practices including use of personal protective equipment to limit spread of disease between humans and nonhuman primates.

Microcystic Meningioma in a Captive Bred African Green Monkey (*Chlorocebus aethiops sabaeus*)

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A group housed 15-y-old, female green monkey was found ataxic, uncoordinated, weak, and falling from side to side. No evidence of trauma or change in body condition was found. Temperature was slightly below normal, but pulse and respiration were within normal limits. Bilateral paresis and occasional opisthotonos-like positioning of the forelimbs were noted. Deficits became more pronounced when the monkey was challenged to move but, while uncoordinated, she was able to jump to her perch. A cisternal CSF sample had blood contamination. CBC and serum biochemical profile results were unremarkable. Empirical treatment with enrofloxacin and dexamethazone was begun. Despite therapy, the animal's condition declined over a fortnight, with progression to right-sided hemiplegia. Euthanasia and postmortem examination were elected. Antimortem CSF had an elevated proportion of nondegenerate neutrophils; consistent with non-specific inflammation. Serum biochemical profile findings included a moderate increase in ALT. Gross examination revealed a white-to-translucent, small, soft, compressive, meningeal mass between cervical vertebrae 3 and 4. Histologically, the mass was infiltrative and the sheets of neoplastic cells formed microscopic cysts. Unilaterally, the ventral and

lateral funiculi had moderate Wallerian degeneration. Microcystic meningioma is an uncommon CNS tumor in animals and people, and causes non-specific, location-dependent signs. To the authors' knowledge, this is the first report of a microcystic meningioma in any nonhuman primate.

Chimpanzee Ocular Herpesvirus

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Dexter, a 22-y-old adult male HIV-positive chimp, suddenly presented with a swollen left eye. By the end of day 2 of conservative anti-inflammatory therapy, his appearance had worsened. The eye was erythematous, edematous, exudative, and obviously painful, and there were open skin lesions on the forehead adjacent to the affected eye. The integrity of the globe appeared to be compromised and there was concern that he would not retain function of the eye. The animal lost interest in all activities (eating, interacting, grooming, etc.) except prodding the affected eye and forehead. Blood work indicated leukocytosis, and elevations in both liver and kidney enzymes. While cultures were pending, the animal was placed on more aggressive therapy, including topical and oral antibiotics and pain medication. Intensive supportive care was provided and consultation was sought. One virologist was able to determine that despite his chronic viral status, based on current CD4 levels, his immune system was intact and should not be a major factor. Following recommendations from a second virologist, a herpesvirus expert, the animal was isolated from his cage mates and the antiherpesvirus drug valacyclovir was added the to the treatment regime. To help identify the causative agent, nucleic acids isolated from swabs (eye and forehead) were subjected to deep sequencing. Metagenomic analysis of the data showed the presence of chimpanzee herpesvirus sequences; this virus is the chimpanzee homolog of herpes simplex virus. The use of next generation sequencing enabled identification of the etiologic agent and formulation of a diagnostic plan in hours rather than weeks. With the addition of antivirals, the animal quickly improved and was eventually restored to full health. Although chimpanzee alphaherpesviruses have been identified before, the sequencing technology was instrumental in rapidly identifying the etiologic agent and placing the animal on the correct treatment regime.

Choosing the "Best Monkey" and Training It for a Diabetes Research Project

M Bushmitz*, S Yosi

BFC

More research projects today gain advantages from training NHP to cooperate with the research team. Trained primates facilitate the research project and make it less expensive. Every study done with trained primates will benefit the animal's welfare, reduce stress, eliminate the need to use chemical restraint, and help to obtain scientifically better results. We learned from experience accumulated over the last few years that monkeys vary in their training performance. We developed a list of criteria which helped to identify the "best monkey" to be trained for a research project. The vicinity of the breeding farm (BFC) to the research institute is the main advantage that allows the Israeli researchers to choose the animal which best suits their research goals. The goal of the project is to choose the "best animal" for a complicated research project which involves multiple interactions with the animal and high numbers of blood testing and injections and to train the NHP to cooperate with the study in the pre and postoperative phase. By using a cooperative animal, you improve the welfare by reducing procedure associated stress. We will try to show you how we chose the "best animal" for a complicated research project trying to find cure for diabetes. We then will show the training process which led to a situation where the animal will cooperate with all the study needs. By choosing the "right" animal, the training period was shorter, easier, and cheaper and improved the welfare of the animals being used. Animals learn to cooperate with 8-10 glucose testing and 3 insulin injections per day for extensive periods. More complicated research can be done using primates without compromising animal welfare during the study. A trained animal facilitates the research as many tests and injections are required daily. The same system is implemented for choosing the right animals for brain research. We hope our practice will help other centers to do the same. Video clips will be used to demonstrate the procedure and results.

Uterus-Like Mass in a Rhesus Macaque (*Macaca mulatta*)

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Endometriosis is defined as the presence of endometrial tissue outside of the uterus and is a common condition in adult female rhesus macaques, particularly those with a history of hysterotomy or other reproductive tract manipulation. Endometriotic foci are comprised of tissue similar to intrauterine endometrium and are often accompanied by variable amounts of fibrous connective tissue and inflammation. Endometriosis with extensive smooth muscle proliferation, also called an endomyometrioma or uterus-like mass, is considered extremely rare in women and, to the author's knowledge, has not been reported in nonhuman primates. We describe a case of endometriosis with smooth muscle metaplasia in a 20-y-old female rhesus macaque. The patient presented to the Surgical Services Unit for a routine protocol related procedure. A large, smooth, globoid, freely moveable mass was palpated in the mid-abdomen. Ultrasonography revealed a cystic structure from which dark brown fluid was aspirated. An exploratory laparotomy was performed and findings included a markedly enlarged uterus, an 8 cm spherical mass in the greater omentum, and 3 additional masses attached to the omentum ranging from 2 to 5 cm in diameter. The masses were excised and ovaries resected based on the intraoperative diagnosis of endometriosis. On microscopic examination, the cystic spaces were lined by columnar epithelium with apical blebs supported by highly cellular spindle cell stroma. Much of the tissue adjacent to this contained abundant smooth muscle interspersed with a densely collagenous stroma. Microscopically, the lesions had similarities to uterus-like masses in women. The pathogenesis of such lesions remains unclear. Both endometriomas and endomyometriomas are hormone-dependent. Common theories are that endomyometriomas may represent a form of stromal metaplasia or originate from a Mullerian system disorder. This is the first report of endometriosis resembling a uterus-like mass in a nonhuman primate.

Nonfunctional Pituitary Hypertrophy after Chronic Administration of a High Potency anti-IgE Monoclonal Antibody

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MedImmune

Elevated serum IgE levels play an important role in certain diseases such as allergic asthma or atopic dermatitis. Biotherapeutics like

omalizumab targeting the IgE axis have shown promising results in clinical practice. A high affinity human IgG1 antibody, targeting soluble IgE with more than 50-fold improved affinity over omalizumab, does not cross-link receptor-bound IgE. This IgG1 antibody binds to cynomolgus monkey, but not rodent, IgE with comparable affinity to human IgE. Repeat-dose studies in cynomolgus monkeys were conducted to establish the safety profile. No noteworthy finding was observed in the IND-enabling 4 wk study up to 150 mg/kg administered once weekly. In contrast, hypertrophy of the pituitary gland was seen in all females at 150 mg/kg (high dose) and in 2 of 3 animals at 50 mg/kg (low dose) in the 6 mo study after once weekly administration. This finding was still present at the end of a 13 wk recovery period in high dose group only, but no downstream functional effects in other endocrine organs were observed. Investigative studies confirmed the absence of hyperplasia and demonstrated that the IgG1 antibody did not bind to circulating female pituitary hormones or to the adenohypophysis. Hypertrophic cells did not stain for FSH, LH, or PrL. Direct interaction of the IgG1 antibody or endogenous IgE with pituitary cell function seems contrary to its pharmacology. Although no clear mechanism of action was identified in these follow-on studies, a NOAEL of 50 mg/kg once weekly was proposed based on (1) lack of functional consequences, (2) low-grade severity of the lesion and (3) slight trend towards recovery.

Pharmacokinetics of Hydromorphone Following Intravenous and Intramuscular Administration in Rhesus Macaques (*Macaca mulatta)*

KR Kelly*, BH Pypendop, KL Christe

Primate Medicine-CNPRC

This study reports the pharmacokinetics of hydromorphone in rhesus macaques (*Macaca mulatta*) after intravenous (IV) and intramuscular (IM) administration. Hydromorphone (0.075 mg/kg) was administered IV as a bolus or IM on separate occasions to healthy, socially-housed, socially-reared, adult, intact male rhesus macaques (n = 4). Blood samples were collected prior to, and up to 10 h, post-administration. Serum hydromorphone concentrations were analyzed with liquid chromatography-mass spectrometry. Compartment models were fit to time-concentration data. A 3 compartment model with input in and elimination from the central compartment best fit IV data; and a 1 compartment model was found to best fit IM data. Following IV administration, the clearance (mL/kg/min) and terminal half-life (h), reported as harmonic mean ± jackknife pseudo-SD (range) were 36 ± 4.63 and 11.1 ± 12.9. The absorption half-life following IM administration was 1.44 ± 1.22 (0.756-6.12) min. Mean IM bioavailability was 96 ± 7.08 (81-109)% [mean ± SEM (range)]. Rhesus macaques maintained concentrations ≥4.00 ng/mL for at least 2 h after IV administration and for 2 to 4 h after IM administration. The disposition of hydromorphone was characterized by a large volume of distribution and moderate clearance. Intramuscular administration resulted in rapid and almost complete drug absorption.

Primates in Medical Research eBook for Tablet

M Bushmitz*

BFC

Electronic books are an exciting new platform. They allow images, sound, and video to create an interactive display that complements the text. The user experience is more immediately engaging and personal than either a website or text book, providing an immersive educational tool which brings the subject to life. The eBook has been used here to provide a new type of text on primates. The eBook, with a potential reader-base of over 60 million, has several advantages over traditional textbooks: a large amount of information can be made available in an easily portable format, they can be updated and revised easily, and they can be distributed freely with no print costs. The book has been developed to help explain the vital role played by nonhuman primates in medical research programs to students, facility employees, stakeholders, and others. Using embedded interactive videos, the following can be more easily communicated in a medium that is more acceptable and tuned to younger people and tech savvy users that might otherwise only see information from those that oppose research. Interviews with animal users, affected patients, doctors, etc., can complement demonstration of techniques that show how animal welfare is maximized in situations such as pair or group housing, enrichment, PRT, play pens, etc. Videos can display animals interacting with peers and people to reflect the environment of caring that predominates in research and breeding farm settings. Animal caretaker interactions with animals, interviews with caretakers, and an explanation of the caging systems and why a particular design is chosen, can further dispel the myths surrounding breeding farm environments. By using this platform, which is very dynamic and can be updated easily, we hope that it will help reshape our communications, adapting them to reach a new generation of readers.

Poster Case Reports

Endotoxemia and Endometrial Necrosis in a Rhesus Macaque (*Macaca mulatta*) Caused by *Escherichia coli*

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Yerkes National Primate Research Center, Emory University

A 12-y-old rhesus macaque presented with lethargy and hypothermia 2 d postpartum. On physical examination, the patient was noted to have pallor, bilateral harsh lung sounds, an enlarged and firm uterus on abdominal palpation, and moderate malodorous vaginal discharge and bleeding. A pelvic ultrasound examination revealed a band of hyperechoic foci within the endometrium. On a vaginal examination, several necrotic tissue fragments were removed and the uterus was copiously lavaged with sterile water. Clinical pathology findings revealed a marked leukopenia with a severe degenerative left shift, anemia, panhypoproteinemia, mild azotemia, and hyperglycemia. The patient was started on broad spectrum antibiotic coverage and aggressive fluid therapy for a suspected septicemia. Despite treatment, the patient rapidly declined and died prior to administration of euthanasia solution. On gross examination, the uterus was severely enlarged with diffuse necrotic mucosa and multiple small cysts. The adrenal glands were also diffusely congested and hemorrhagic. Histopathologic examination revealed severe endometrial necrosis and mild endometritis, and routine cultures of the blood, heart, and tissues were positive for *Escherichia coli*. The clinical history and pathologic findings were consistent with endotoxic shock, resulting in acute death.

Aural Hematomas

A Saucedo*, PR Morales, JL Wagner

The Mannheimer Foundation

The present work represents a retrospective study of aural hematomas in macaque species group housed in outdoor enclosures in a facility located in South Florida. Aural hematomas are described in

humans as cauliflower ear, hematoma auris, perichondrial hematoma and traumatic auricular hematoma. In humans, this condition is related to sports injuries, but can at times be related to trauma or an infection to the ear lobe. Auricular hematomas develop in the concave surface of the pinnae in dogs, cats and pigs, due to head shaking or ear scratching due to pruritus. The treatment of choice varies slightly amongst the different species. To the authors' knowledge no cases have been published pertaining to this common observation noted in macaques of all ages.

Myxoid Liposarcoma in a Cynomolgus Macaque (*Macaca fascicularis*)

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Merck Research Laboratories

A 5-y-old male cynomolgus macaque (*Macaca fascicularis*), that had not been on study since April 2011, presented acutely for severe lethargy and pale mucous membranes. The animal was sedated with ketamine for physical exam and was cold to the touch (rectal temperature 92.8 °F) with a hard, taut, and distended abdomen. Gastric dilatation was suspected and a gastric tube passed, releasing a large amount of digested feed and fluid. Despite relief of gastric pressure the animal was very painful upon abdominal palpation, vocalizing multiple times during the exam. Dexamethasone (1 mg/kg IM), buprenorphine (0.01 mg/kg IV), and enrofloxacin (5 mg/kg IM) were administered while options were discussed with the PI. The animal bloated a second time, and due to the poor prognosis the PI declined surgical intervention, electing euthanasia. At necropsy, the stomach was grossly distended with diffuse mucosal hemorrhage and suspected necrosis. A large (5 × 3.5 cm) mass was found over the right kidney and adhered to the liver and diaphragm as well as encompassing the right floating rib. The mass was firm and covered with a clear viscous material. The cut surfaces had a fatty appearance with multiple coalescing, 0.4 to 0.5 cm yellow to tan nodules. Histomorphologically, the nonencapsulated mass was bordered on one surface by skeletal muscle and was characterized by multiple coalescing nodules separated by a fibrovascular stroma. The nodules were composed of stellate to polygonal cells arranged in tightly to loosely packed sheets separated by a pale basophilic staining material (mucin). The cells had scant eosinophilic cytoplasm containing variable-sized, clear vacuoles, and 1 to 3, round to oval nuclei. Mitotic figures were not observed. Within the stroma were slight infiltrates of lymphocytes, plasma cells, macrophages admixed with hemorrhage. The histomorphologic features of the mass are consistent with a myxoid liposarcoma. Currently, this tumor type has only been reported once in a nonhuman primate.

Ovarian Carcinoma in a Cynomolgus Macaque

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Covance Laboratories

During a routine reproductive evaluation of a 6-y-old female cynomolgus macaque (*Macaca fascicularis*), a mass was noted on palpation of the caudoventral abdomen. Ultrasonic evaluation revealed a partially cystic or lumenal, irregularly shaped structure with mixed echogenicity peripheral to the hypoechoic center of the mass. There were multiple smaller areas of hyperechogenicity close to the periphery of the cystic portion of the lesion. The animal was otherwise in good body condition. This finding was incidental, and radiographs of the thorax and abdomen were unremarkable. As the animal was part of the developmental and reproductive toxicology program at our facility, and would have been unsuitable for study in this program, euthanasia and necropsy were elected. At necropsy, the mass, measuring 6.0 × 6.0 × 5.0 cm, was found to involve the right ovary. A morphologic diagnosis of ovarian carcinoma was determined. Ovarian carcinoma is known to be a significant tumor type in humans. This diagnosis, however, is so far unreported in the cynomolgus macaque, and this appears to be a novel or unrecognized tumor type for this species.

The Normal Environment of Healthy Cycling Cynomolgus Macaques Appear Equivalent to Bacterial Vaginosis in Women

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Evaluating strategies for the prevention and control of HIV transmission in humans remains a top priority, since new infections predominantly affect women in limited resource settings. We are using cynomolgus macaques (CM) to test a vaginal microbicide strategy to block mucosal transmission of simian/human immunodeficiency virus (SHIV) that uses recombinant human broadly neutralizing HIV monoclonal antibodies (Mabs) produced in *Nicotiana benthamiana*. CMs were chosen because their reproductive cycle is continuous which is similar to humans as opposed to other macaque species whose cycle is seasonal. In order to determine the usefulness of the CM as a model for vaginal transmission and treatment of SHIV, we first characterized the composition of the vaginal environment of healthy, nonsexually active adult CM monkeys. Fifteen sexually cycling female CM were surveyed over a 12-wk period for vaginal microflora, Nugent score, vaginal pH, Amsel criteria, glycogen content, cervical vaginal lavage and determination of the menstruation cycle. Baseline evaluation of the CM monkey demonstrated that similar to other macaques, their vaginal pH varied from 5.5 to 9, without evidence of inflammation or abnormal discharge. Nugent scores showed a paucity of lactobacilli with scores between 8 and 10, indicative of a vaginal environment comparable to those of women with bacterial vaginosis (BV), a state associated with increased susceptibility for HIV transmission. We propose that in addition to being a valid model of HIV transmission, the CM monkey may also be considered as a model for BV in women. Additional studies to further investigate the findings of BV in CM are needed.

Enamel Hypoplasia in Cynomolgus Monkeys

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Covance Research Products

One-hundred and twenty cynomolgus macaques were imported from Cambodia to our quarantine facility in May 2013. There was an equal distribution of males and females approximately 3 y old. Upon physical examination during quarantine, 74 animals were observed to have some degree of enamel hypoplasia of the permanent dentition. Incisors were most commonly affected. Grossly, the enamel had a multifocal pitted to coalescing pattern of loss, giving the teeth a mottled and uneven appearance, often with a yellow to brown discoloration. The lesions do not appear to be from wear as they are seen mainly on nonocclusive surfaces of the teeth. Our most likely differential diagnoses include nutritional imbalances, toxicities and/or viral infections of either the pregnant dams of the affected monkeys or the monkeys themselves during infancy. Detailed information from the supplier that would help determine the

possible etiologies was not available. Two animals, one affected and one unaffected, were necropsied to help determine the most likely cause of the enamel defect and whether other systems were affected. Histopathology results and possible etiologies will be presented.

The Efficacy and Safety of Antithymocyte Globuline (ATG) to Treat Acute Uterus Transplant Rejection in 2 Baboons (*Papio hamadryas*)

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The Mannheimer Foundation

Uterus transplantation may become a surgical option for women with a diseased or absent uterus. Without a functioning uterus, embryonic implantation cannot occur and females are rendered infertile (uterine factor infertility or UFI). During the past several years, various species have served as an animal model to refine uterine transplantation techniques. Recently, the baboon model has become particularly useful due to commonalities in uterine anatomy and reproduction processes. However, acute and chronic graft rejections may complicate the post-transplant course of the transplant. These are still major challenge for both women and their nonhuman primate counterparts. This study considers the safety and efficacy of antithymocyte globulin (ATG) to prevent and treat acute uterine graft transplantation rejection in the *Papio hamadryas* baboon model. Subjects included 2 captive born adult female baboons (6 to 7 y old) that underwent uterus transplantation (Utx) at The Mannheimer Foundation. Both subjects received ATG (10 mg/kg) via IV infusion for 2 d prior to uterine transplantation surgery. ATG was provided as an induction immunosuppressionant to reduce circulating T- cells lymphocytes and delay or prevent transplant rejection. ATG was used in combination with conventional immunosuppressant treatments (Tacrolimus 1 mg/kg, administered intramuscularly, with dose adjusted to achieve blood target levels) and steroids. A week later post-transplantation, severe acute rejection was observed in the 2 animals, diagnosis was suspected by clinical findings (visually and by palpation) and later proven by biopsy. Subjects subsequently underwent additional ATG treatment via IV infusion (10 mg/kg) during 10 d. Response to treatment was assessed by endoscopic examinations and sampling of the graft (biopsies) and size of uterus. One subject responded to treatment as documented by histopathology reports (serial graft biopsies) and the clinical findings (reduction of the size of the graft). Results suggest that ATG may be a safe and effective treatment for the prevention and treatment of uterus graft transplant rejection. The study was reviewed and approved by The Mannheimer Foundation Institutional Animal Care and Use Committee.

The Use of Budesonide for the Treatment of Chronic Diarrhea in Macaques

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Chronic idiopathic enterocolitis (CIC) is a common cause of morbidity and mortality in macaques, affecting up to 15% of those housed in captivity. CIC is generally characterized histologically by an infiltration of lymphocytes and plasma cells in the small intestines, colon, and cecum, but is not a homogenous disorder and shares features with several human inflammatory bowel conditions. Of these, it has been reported that similarities to chronic lymphocytic-plasmacytic colitis (CLPC) are most common among cases of CIC. Budesonide is the most commonly used drug to treat CLPC in humans and has both fewer systemic side effects and a lower relapse rate after cessation of treatment than other corticosteroids such as prednisone. We will report the results of our clinical use of budesonide in clinically defined cases of CIC in indoor/outdoor housed breeding groups of rhesus (*Macaca mulatta*) and pigtailed macaques (*Macaca nemestrina*).

A Novel Technique for Collection of Cervical Cells from Rhesus Macaques Assigned to SIV Research Studies

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There has been increased interest in the pathophysiology and immune response to heterosexual HIV transmission via the intravaginal route. There are information gaps that need to be filled concerning the viral targets and immune response that occurs locally within the vagina. Nonhuman primates experimentally infected with SIV are a well characterized animal model for studying the mechanisms of prevention, infection and treatment of HIV in humans. Although studies have been done that describe the macaque vaginal environment current data suggest that the cervix with its cuboid single cell epithelium is a more likely portal of entry. To study this compartment, a novel cytobrush collection of endocervical cells was tested in healthy rhesus monkeys at Yerkes National Primate Research Center. The nontraumatic technique was used to collect sequential samples to be analyzed by multicolor flow cytometry, to characterize the subset of hematopoietic endocervical cells present at various stages of the estrous cycle. In addition the technique was used to quantify the ability of a primatized anti-α4β7 monoclonal antibody administered parenterally to reach cervical CD4 and CD8+ T cells, as a preliminary to test this treatment in the context of simian immunodeficiency virus infection. Among over 200 cervical brush samples collected, 54% provided cell samples that generated useful data via flow cytometry. Failure to obtain the expected cellular samples were tied to menses, contamination with vaginal cells, lack of detectable cells or lack of CD45+ hematopoietic cells in the collection. Nevertheless, to our knowledge, this constitutes the first report of cervical brushes collected from rhesus macaques for the purpose of evaluating the frequencies of SIV targets at this portal of entry. We anticipate this technique to markedly facilitate vaginal transmission studies including microbicide investigations using nonhuman primates.

Refinements to Maximize Blood Sampling for IV-GTT Procedure in Cynomolgus Macaques (*M. fascicularis*)

KN Poullé*, P Ramlochun, M Rakotomaharo

Bioculture

All those who have been involved in collecting blood samples at the different time points during IV-GTT know how much of a struggle it can be sometimes to perform this procedure. The smaller size of a cyno (compared to a rhesus); the higher rate at which they metabolise ketamine and several other factors, do sometimes make it quite a challenge to sample repetitively blood from one same catheter, while minimizing the amount of haemolysis in the serum. The poster will describe a few simple steps that would help all those involved in IV-GTT to maximize their chances in getting all the samples in due time; at appropriate volumes; without having to "struggle" a lot (the major cause delay and haemolysis).

A Practical Method to Calculate Maximum Blood Collection Volume from Obese Rhesus Macaques Based on Predicted Lean Body Weight

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Yerkes National Primate Research Center

Veterinary recommendations for the safe withdrawal of blood from laboratory animals are based on total blood volume expressed as a percentage of body weight. The most accurate estimate of total blood volume is based on lean body weight; however estimating lean body mass in obese rhesus macaques with large abdominal fat pads and other subcutaneous fat can be difficult in a clinical setting. As a result, blood collections based on actual body weights in these macaques can lead to over-withdrawal of blood and development of anemia. The purpose of this report is to present a practical method to estimate safe blood sample volumes from obese rhesus macaques based on predicted lean body weight instead of actual body weight. Fat and lean body mass data obtained from dual-energy x-ray absorptiometry (DEXA) scans are reliable measures of body composition in macaques. A linear regression model approach was used to analyze fat and lean body mass data from >150 DEXA scans of adult female macaques. To determine maximum blood sample volume, standard institutional guidelines can still be followed that typically permit a percentage of body weight for calculating maximum blood volumes to be collected over a defined time period (for example 7.5% or 10% of body weight every 4 wk). Linear regression equations can be used to estimate fat mass when such measures are not available that, in turn, can be used to calculate lean body weight by subtracting predicted fat mass from actual body weight. Predicted lean body weight can then be used to calculate maximum collection volumes based on individual institutional guidelines. Supported by R01 MH081816, HD046501, and OD P51OD11132.

Blood Collection and Plasma Biochemistry Values in the Brown Mouse Lemur (*Microcebus rufus*)

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Mouse lemurs (*Microcebus* spp.) are nocturnal prosimians endemic to Madagascar and include the world's smallest primates. Their basic ecology, behavior, and speciation are areas of active study in wild populations. Additionally, captive breeding colonies of the gray mouse lemur (*M. murinus*) may offer excellent animal models of aging and Alzheimer disease. Blood samples can provide useful information about health, genetics, and hormone status in both wild and captive individuals, but may be difficult to obtain in small animals. This study describes a blood collection method in the brown mouse lemur (*M. rufus*) from the medial saphenous vein, adapted from techniques commonly used in laboratory rats and mice. This method reliably provided up to 250 µL of blood from mouse lemurs ranging 40 to 60 g in body weight. No adverse effects were observed and this technique did not require sedation of the animals. Blood samples were analyzed using a handheld clinical analyzer and biochemical values were comparable to published results from other lemur species. This is the first report of blood biochemical values from a free-ranging mouse lemur population. Venipuncture of the medial saphenous vein provides a safe blood collection technique in the mouse lemur and may be useful for other small primates.

Effect of Different Injectable Anesthetic Combinations on Blood Gas Values in Baboons (*Papio cynocephalus anubis*)

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Anesthesia or sedation is commonly required when collecting biologic samples from baboons (*Papio cynocephalus anubis*). Anesthetic agents are known to cause a decrease in pulmonary function and central respiratory drive leading to a change in arterial blood gas (ABG) values. To determine which anesthetic protocol disturbed respiratory function the least, we measured ABG using an iSTAT analyzer before and after administering various combinations of anesthesia to baboons of about 3 y of age and both sexes. Blood collection prior to administration of anesthesia was accomplished via an arterial vascular access port (VAP). The tip of the catheter was positioned in the abdominal aorta and the port placed subcutaneously on the medial aspect of a distal hind limb. We sedated the baboons with several different combinations of anesthetics including ketamine (10 mg/kg) in combination with acepromazine (0.5 mg/kg) (KA), ketamine alone (10 mg/kg) (K), and tiletamine–zolazepam (5 mg/kg) (T) all given intramuscularly. Depth and duration of anesthesia, temperature, respiratory rate, and ABG values were collected at time points before and after administration of anesthetics. All anesthetic combinations had a similar effect on ABG levels. For K, pCO_2 increased from a baseline of 34.1 to 44 at the 10 min post anesthesia time point ($P < 0.01$, $n = 7$). For KA, pCO_2 increased from 34.7 to 42.3 ($P < 0.01$, $n = 8$). For T, pCO_2 increased from 36.3 to 46.5 ($P < 0.01$, $n = 3$). For all anesthetic combinations, pCO_2 gradually declined after the 10 min time point but was not back to baseline by the last time point at 30 min. pO2 levels were suppressed significantly at the 10 min time point for all combinations of anesthesia. For K, pO_2 decreased from a baseline of 80.6 to 68.6 10 min post anesthesia ($P = 0.01$, $n = 7$). For KA, pO_2 decreased from 85.5 to 74 ($P < 0.01$, $n = 8$). For T, pO_2 decreased from 86.3 to 64 ($P = 0.01$, $n = 3$). Levels did not change significantly from there for the duration of the study. In conclusion, arterial pO_2 and pCO_2 levels were significantly altered after several different combinations of injectable anesthesia were administered to baboons. Suppression of ventilation persisted for an unexpectedly long interval after induction of anesthesia. Values for ABG remained outside normal ranges even after baboons were starting to recover from anesthesia.

PET/CT and MRI Imaging in Animal Biosafety Levels 3 and 4: Challenging Primate Models

WR Elkins, M St Claire*

NIAID/NIH

Central to the core mission of the NIAID's biocontainment facilities is the use of hospital tools, including Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and Positron Emission Tomography (PET), to systematically evaluate the pathogenic processes and clinical course of disease in animal models exposed to emerging infectious pathogens. A key goal of our work is to correlate the transmission, virulence, and invasiveness of high consequence microbial agents with clinical tests and diagnostic imaging. Nonhuman primates in particular present unique challenges in ABSL3 and 4 environments. Highly compromised animals frequently undergo multiple imaging procedures using radioactive materials for time periods of up to 7 h, presenting radiation safety, anesthesia and patient support issues. Prevention of cross-contamination between animals on different studies using the same space is a constant battle when working with dangerous, exotic microbial agents. Personnel safety and accident prevention

must also be considered in the high containment setting. The techniques, equipment, and procedures presented describe novel methods for addressing the challenges of imaging in biocontainment laboratories.

Development of an Indirect ELISA for the Detection of *Burkholderia pseudomallei*

A Leon*, G Khara, K Brittingham

BioReliance

Burkholderia pseudomallei (Bp) causes melioidosis, a severe life-threatening bacterial infectious disease that affects both humans and animals. Bp is a gram negative, aerobic bacterium found in the soil throughout Southeast Asia and northern Australia. Screening nonhuman primates that are imported from Southeast Asia for Bp exposure has become critical to ensuring naïve animals are used in Bp research. This will minimize the potential impact of pre-existing antibodies on the survival of Bp challenged animals while minimizing results' variability and reducing the number of animals required for developing melioidosis therapeutics. Currently, there are no commercial assays for Bp exposure detection. We developed an indirect ELISA assay to detect the presence of serum IgG antibodies reactive to Bp for screening of nonhuman primates. The assay was developed using detergent lysed Bp bacteria. Before use in the assay, detergents were removed from the lysate and inactivation of bacteria was verified before removal from a BSL3 laboratory. Uninoculated growth media was used as control antigen. Positive samples were obtained from nonhuman primates that were experimentally infected with Bp. Naive samples were obtained from animals raised in China and U.S. The appropriate concentration of the components was determined by cross-titration of antigen and control antigen using a positive control antibody produced in vaccinated goats. Comparisons between the results obtained from positive and naïve samples indicated that the optimal coating antigen concentration was 100 ng/mL. Proof of principle was demonstrated by coating plates with 100ng/mL of Bp antigen and control antigen, and testing 19 positive and 40 naïve samples. The assay distinguished between positive and negative samples at a preliminary cut-off value of OD405=0.6. The assay was qualified by testing 19 positive and 40 naïve US born nonhuman primate samples in triplicate. The assay is 100% sensitive and 93% specific and has a confirmed cut-off value of OD405=0.6. To date, this assay has been used to screen rhesus macaques imported from vendors in Southeast China resulting in rejection of 19.3% of the animals from being used in Bp research.

Trends in the Use of Nonhuman Primates for Research in North America, 2010-2012

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The North American research community faces social and economic challenges to nonhuman primate (NHP) importation that could reduce the number of NHPs available to meet research needs. How such limitations would affect specific biomedical research areas is not well understood. The Association of Primate Veterinarians (APV) and the Centers for Disease Control and Prevention (CDC) surveyed the APV membership regarding North American institutional use of NHPs in biomedical research from 2010 to 2012. The purpose of the survey was to determine the number and species of imported NHPs maintained at facilities, current research uses of NHPs in biomedical research, and perceived trends in NHP research. Of 149 respondents contacted, 33 (22%) replied, representing a diverse sample of facility sizes and types. Macaques, particularly cynomolgus and rhesus, were the most common species housed and imported during this period. The most common uses reported for NHPs included pharmaceutical research and development, and neuroscience, neurology or neuromuscular disease research. Preclinical safety testing and cancer research relied more on imported NHPs, while research on aging or degenerative disease, reproduction or reproductive disease, and organ or tissue transplantation more commonly used domestically bred NHPs. The results of this survey contribute to an improved understanding of research uses for imported NHPs in North America and might assist with estimating the potential impact of any future changes in NHP accessibility for research purposes. Ensuring that adequate numbers of NHPs are available for critical biomedical research remains a pressing concern for the North American biomedical research community.