

**PROCEEDINGS**

**2019**

**51ST AMERICAN ASSOCIATION OF  
ZOO VETERINARIANS**

**Annual Conference**

**Saint Louis, Missouri USA  
28 September - 4 October, 2019**

**CHARLOTTE KIRK BAER  
PROCEEDINGS EDITOR**

## CONTINUING EDUCATION

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# AMERICAN ASSOCIATION OF ZOO VETERINARIANS

*"Dedicated to wildlife health and conservation"*

581705 White Oak Road • Yulee, Florida, 32097 • 904-225-3275 • Fax 904-225-3289

Dear Friends and Colleagues,

The Gateway to the West, River City, The Lou (that's LOU not Loo!). It is my pleasure to welcome you to St. Louis, Missouri, USA for the 51st American Association of Zoo Veterinarians (AAZV) Annual Conference, concurrent with ExoticsCon, the Zoo and Wildlife Nutrition Foundation/Association of Zoos and Aquariums Nutrition Advisory Group (NAG), and Association of Fish Veterinarians (AAFV). On behalf of AAZV, I would like to extend a warm welcome to the members, the members of our fellow organizations, and our international colleagues in attendance. Congratulations to the AAZV International Scholarship awardees and a very special welcome to our new members, first-time conference attendees, and students.

Our Scientific Program Committee has put a great deal of time and energy into preparing a dynamic and educational program that reflects current knowledge and methodologies across our global community. I would like to acknowledge the American College of Zoological Medicine for providing continuing education credits for the conference.

Conference attendees will be treated to some midwestern hospitality from our hosting institution, the Saint Louis Zoo. Thanks to our hosts Luis Padilla, Chris Hanley, Sathya Chinnadurai and the staff and volunteers from their institution for opening their facility to us and making us feel welcome.

This conference is a product of the hard work of our conference planning team, Rob Hilsenroth, Adine Nicholson, Julie Best, and Julie Fazlollah, as well as contributions from our student volunteers, who perennially ensure the successful planning and implementation of this event.

Most importantly, I want to thank you the membership for your dedication to our profession and support of our organization. AAZV is a diverse and energetic organization because of your continued membership, contributions, and professional service. Our Wild Animal Health Fund and granting capacity continues to grow and the Journal of Zoo and Wildlife Medicine, celebrating its 50th year in print, remains the premier source of scientific and clinical information for our field. In all that you do, AAZV members never fail to challenge, amaze, and inspire me with their passion, perseverance, knowledge, curiosity, and commitment.

With respect and appreciation,

Julie Napier  
President, American Association of Zoo Veterinarians



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Dear Colleagues,

Welcome to St. Louis for the 51<sup>st</sup> American Association of Zoo Veterinarians (AAZV) Annual Conference! On behalf of the Scientific Program Committee (SPC), we are delighted to share this year's thoughtfully crafted program with you. We want to thank abstract authors, session chairs, workshop instructors, invited speakers, the Executive Committee, and the AAZV office staff without whom this program would not be possible. This conference will provide a great opportunity to network with colleagues from ExoticsCon, the Association of Zoos and Aquariums Nutrition Advisory Group (NAG), and Association of Fish Veterinarians (AAFV). We anticipate an exciting and busy week and have tried to our best to align schedules whenever possible.

This year's offering of 11 workshops is varied and practical. We have worked closely with our local host, the Saint Louis Zoo, to highlight many topics unique to the institution and area. Participants will have the opportunity to learn about hellbender medicine, small gazelle handling and medicine, canid restraint and anesthesia, urban wildlife and conservation medicine techniques, and reproductive management. In addition, we collaborated with NAG to bring you a special workshop on nutrition topics. We continue to bring leadership training opportunities with a leadership workshop taught by our AAZV colleagues.

The line-up of program talks is robust and promises to be inspiring to our diverse audience. From over 200 submissions, the session chairs and SPC have carefully selected the talks and posters that you will be viewing this week. We are continually awed by the amazing work all of you do, and this selection process is far from easy. Due to continued popularity and importance, a session is devoted to the topic of welfare. Timely sessions on imaging and One Health will be further highlighted by the addition of inspiring featured speakers on these subjects. This year we are also excited to offer continuing education that should expand upon some of the soft skills of the profession, including special talks on financial health and online literature searching skills. At the start of the week, veterinary students will have the opportunity to participate in the first student-only master class on zoo and wildlife pathology. The week will close with exceptional master class talks on integrative medicine for geriatric zoo animals, respiratory complications in anesthesia, and how to get the most out of pathology. In total we are able to offer 22.75 lecture credit hours and 16 workshop credit hours. Thank you to the American College of Zoological Medicine for approving this CE.

As always, we are eager for feedback on the scientific program. We strive to create a well-rounded program that serves the broad interests of our membership. At the conclusion of the conference, please take a few minutes to complete the conference survey to tell us your thoughts and ideas. We can't emphasize enough that your abstract submissions, and your comments and suggestions on the conference evaluation truly shape our future program content. We hope that this week is thought provoking, inspiring, and gives you a chance to reconnect with the themes and people of our profession.

Best wishes,

Kristen Phair, DVM, Dipl ACZM  
Chair, AAZV Scientific Program Committee  
Director of Veterinary Services, Phoenix Zoo

# American Association of Zoo Veterinarians 2019 Scientific Program Committee

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## PRACTICAL GUIDANCE FOR OPTIMIZING QUALITY OF COMPUTED TOMOGRAPHY STUDIES IN ZOOLOGICAL SPECIES

*Marina Ivančić, DVM, Dipl ACVR\* and Michael J. Adkesson, DVM, Dipl ACZM, DipECZM (Zoo Health Management)*

*Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513 USA*

### **Abstract**

Use of cross-sectional imaging, particularly computed tomography (CT), is on the rise in clinical zoological medicine. Advanced imaging technologies such as CT necessitate a degree of technical knowledge and planning that is more complex than radiography or conventional ultrasonography. This is true for all domestic animals, but the more complicated logistics of zoological patient handling demand maximal preparation, coordination, efficiency, and expertise. Whether scans are performed in-house at a zoo, or externally at a referral center or human hospital, practical knowledge of CT acquisition parameters by zoo and aquarium clinicians can greatly enhance the diagnostic quality of a study. Optimal positioning is critical, based on the species, anatomical region of interest, disease process, and anesthetic plan. Choices regarding slice thickness, scan field-of-view, exposure settings, reconstruction algorithms, and order of body region assessment all affect image interpretation. Photon starvation considerations with large animals can often be overcome by adjusting scan parameters and/or the geometric alignment of limbs. Timing of image acquisition following contrast administration, choice of contrast media (concentrations, pharmacology), and comprehension of the utility and limitations of contrast usage are all part of careful planning. Recognition and prevention of artifacts are critical in deciding whether scans need to be repeated or can be corrected. Application of CT-guided sampling may also impact scan considerations. Generation of multiplanar and minimum/maximum intensity projections after a scan can improve lesion identification. Practical guidelines for clinical zoo veterinarians detailing planning and execution of CT studies are presented.

**Key words:** Computed tomography, CT, diagnostic imaging, radiology, zoological species

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## SELECTION, ACQUISITION, AND INCORPORATION OF A PORTABLE COMPUTED TOMOGRAPHY UNIT INTO TWO ZOOLOGIC MEDICAL PRACTICES: PROS, CONS, AND ASSOCIATED COSTS

*Benjamin N. Nevitt, DVM, Dipl ACZM,<sup>1\*</sup> Matthew E. Kinney, DVM, Dipl ACZM,<sup>2</sup> and Patrick J. Morris, DVM, Dipl ACZM<sup>1</sup>*

*<sup>1</sup>San Diego Zoo, San Diego, CA 92112 USA; <sup>2</sup>San Diego Zoo Safari Park, Escondido, CA 92027 USA*

### **Abstract**

As the use of computed tomography (CT) in zoologic veterinary medicine has become more common, many zoologic practices are still limited to saving advanced imaging for high-profile animals or difficult cases, as transport to offsite facilities with CT comes with many challenges. Safety, logistics, and convenience are all more attainable if CT technology is on-site. However, the upgrades and cost required to install a traditional CT unit in an existing hospital are significant.

In 2017, veterinarians from San Diego Zoo Global (SDZG) began the process of purchasing a CT for both the San Diego Zoo and San Diego Zoo Safari Park that would provide high-quality, diagnostic CT scans for the variety of species in both collections, while also not requiring major renovations to either existing hospital. A systematic approach was used to evaluate potential scanners, focusing on study quality, ability to accommodate the collection's species diversity, integration with existing facilities, and ease of operation. SDZG was able to purchase and install two portable CT scanners (Bodytom®, NeuroLogica Corp, subsidiary of Samsung Corp., Danvers, MA, USA), which fit the criteria for the needs of the organization.

This scanner is a 32-slice CT scanner with a large bore (85 cm gantry and 60 cm field of view). It is battery-powered, which allows use in multiple rooms of each hospital. The unit can charge from a standard electrical outlet and is, itself, lead-lined, requiring minimal facility modifications. It weighs 3,510 pounds and requires a firm, flat service for operation. The scanner has intuitive operation, allowing many veterinarians and veterinary technicians at each facility the ability to operate the scanner. However, the scanner does have limitations and the cost for the units and service was significant.

Current applications, costs, future projects, and other advantages and disadvantages of this portable CT compared to traditional CT scanners will be discussed further.

**Key words:** Advanced imaging, computed tomography, portable CT

### **ACKNOWLEDGMENTS**

San Diego Zoo Global graciously thanks the Alt Estate for its support, which allowed for acquisition and installation of a CT scanner at both the San Diego Zoo and the San Diego Zoo Safari Park. The authors thank the staff of both hospitals for their efforts to learn and integrate this technology into their daily practice.

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## RADIOGRAPHIC ANATOMY AND BARIUM SULFATE CONTRAST STUDY OF THE GASTROINTESTINAL TRACT OF EASTERN BOX TURTLES (*Terrapene carolina carolina*)

*Emma L. Houck, DVM,<sup>1\*</sup> Eli B. Cohen, DVM, Dipl ACVR,<sup>2</sup> Mandy Womble, PhD,<sup>1</sup> Gregory A. Lewbart, MS, VMD, Dipl ACZM, DipECZM (Zoo Health Management),<sup>1</sup> and Olivia A. Petritz, DVM, Dipl ACZM<sup>1</sup>*

<sup>1</sup>*Department of Clinical Sciences, North Carolina State University College of Veterinary Medicine, Raleigh, NC 27606 USA; <sup>2</sup>Department of Molecular Biomedical Sciences, North Carolina State University College of Veterinary Medicine, Raleigh, NC 27606 USA*

### Abstract

This study describes the normal radiographic anatomy, transit, and emptying times of the gastrointestinal tract in healthy eastern box turtles (*Terrapene carolina carolina*) administered 15 ml/kg barium sulfate diluted to 30% weight per volume via orogastric gavage. Three-view radiographic series (vertical beam dorsoventral, horizontal beam latero-lateral, and horizontal beam rostrocaudal views) were obtained prior to contrast administration, and following contrast administration at 0, 20, 40, 60, and 90 min, 2, 4, 8, 12, and 24 hr post administration, and every 24 hr thereafter until all contrast was eliminated. Vertical beam dorsoventral and horizontal beam latero-lateral views were of excellent quality to identify gastrointestinal structures. The horizontal beam rostrocaudal view immediately post-contrast administration provided gastric and pyloric identification but had lesser diagnostic use at later time points due to anatomic superimposition. The gastrointestinal tract was composed of a tubular stomach, a pyloric sphincter near the midline, a duodenum with a cranial flexure in the right cranial coelomic cavity, small intestines within the right coelom, a small cecal bulb, and a transverse and descending colon. Contrast media entered the large intestine by 24 hr in all turtles, and a pyloro-colic indentation was noted at the proximal descending colon. The large intestinal emptying was highly variable due to the interindividual variability of contrast sequestration within the cecal bulb. This is the first study to detail the gastrointestinal anatomy, transit, and emptying times in healthy eastern box turtles, and to evaluate the horizontal beam rostrocaudal view for gastrointestinal contrast studies in any chelonian.

**Key words:** Barium sulfate, Chelonia, contrast-enhanced, eastern box turtle, radiography, *Terrapene carolina carolina*

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## ULTRASOUND-GUIDED ENDOSCOPIC TRANSCERVICAL ENDOMETRIAL BIOPSY IN ZOO SPECIES

*Gwen Jankowski, DVM, MS, Dipl ACZM,<sup>1</sup> Anneke Moresco, DVM, PhD,<sup>1</sup> and Sushan Han, DVM, PhD, DACVP<sup>2\*</sup>*

*<sup>1</sup>Denver Zoo, Denver, CO 80205 USA; <sup>2</sup>Colorado State University, Fort Collins, CO 80523 USA*

### Abstract

Endometrial biopsy and histology are required for diagnosis of conditions that can lead to infertility such as endometrial hyperplasia, endometritis, and neoplasia. A novel technique for ultrasound-guided endoscopic transcervical endometrial biopsy (TCEB) is described in four non-human primate species and a southern tamandua (*Tamandua tetradactyla*). A 1.7-mm or 2.7-mm rigid endoscope (KARL STORZ Endoscopy-America Inc., 2151 E Grand Ave, El Segundo CA 90245 USA) is inserted through the cervical os for visual examination of the endometrium. Transabdominal ultrasound guidance is used to biopsy the endometrium with endoscope biopsy forceps. Ultrasound is used to evaluate for complications of the uterus and peri-uterine abdomen, such as hemorrhage. Rigid endoscopy TCEB confirmed processes such as endometritis, endometrial polyp, and normal cycling. Diagnostic biopsies were obtained in primates as small as a capuchin monkey (*Cebus capucinus*), though crush artifact using the 1.7-mm scope limited subtle cellular details. Subsequent histologic evaluation of three biopsied uteruses showed no lasting lesions secondary to biopsy. Although no complications with this technique occurred in these cases, potential risks include excessive uterine hemorrhage, infection, or possibly uterine perforation. Therefore, the use of sterile technique and ultrasound guidance are recommended. Current population sustainability challenges for many of AZA's breeding programs may benefit from this technique to diagnose subclinical conditions at an earlier, more treatable stage.

**Key words:** Endometrial, endoscopy, primate, tamandua, ultrasound

### ACKNOWLEDGMENTS

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## SPINAL OSTEOARTHRITIS IN GERIATRIC PRIMATES: A PROPOSED RADIOLOGIC SCORING SYSTEM

*Emmanuelle A. Furst, DVM,<sup>1\*</sup> Sophie Dennison, BVM&S, Dipl ACVR,<sup>2</sup> and Cora L. Singleton, DVM, Dipl ACZM<sup>3</sup>*

*<sup>1</sup>All Creatures Animal Hospital, East Amherst, NY 14051 USA; <sup>2</sup>TeleVet Imaging Solutions, PLLC, Oakton, VA 22124 USA; <sup>3</sup>San Diego Zoo, San Diego Zoo Global, San Diego, CA 92101 USA*

### Abstract

Osteoarthritis (OA) is a leading cause of disability and reduced quality of life in humans. Prevalence of lumbar spine OA in humans ranges 40-85%, with 80% of Americans experiencing lumbar pain during their lifetime.<sup>1</sup> A similar trend of high prevalence and associated pain is likely paralleled in nonhuman primates under human care.

This study proposes a standardized quantitative index for objective radiologic assessment of osteoarthritic changes of the spine. Ideal joint positioning for different imaging modalities is defined. Four key radiologic parameters are selected for evaluation of the spine: intervertebral disc space narrowing, vertebral endplate sclerosis, osteophyte formation, and articular facet changes.<sup>2</sup> Focus is placed on spinal structures with adequate nerve supply, capable of generating pain. Each intervertebral space (IVS) in cervical, thoracic, and lumbar spine is scored for each of these four parameters on a five-point scale. Total score for each IVS is used to classify the joint as having absent, mild, moderate, severe, or profound osteoarthritis. Scores for each IVS can be summed for a regional OA score.

A standardized OA grading system will facilitate thorough evaluation and documentation of joint changes over time. Scores for spinal OA can be combined with OA scores for shoulders, elbows, hips, and knees to give an overall assessment of degenerative changes that may cause pain and impact mobility. With heightened awareness of indicators of early OA, targeted medical, behavioral, environmental, and physical interventions may improve animal comfort and welfare and ideally slow the progression of this debilitating disorder.

**Key words:** Geriatric, nonhuman primate, osteoarthritis, radiologic, spine, welfare

### ACKNOWLEDGMENTS

The authors thank Dr. Megan Freeman and Dr. Melissa King-Smith for their development of osteoarthritis scoring systems for the shoulders/elbows and hips/knees, respectively.

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## A REVIEW OF PARTURITION PARAMETERS OF TWO SPECIES OF CAPTIVE RHINOCEROS: WHITE RHINOCEROS (*Ceratotherium simum*) AND GREATER ONE-HORNED RHINOCEROS (*Rhinoceros unicornis*)

*Emily E. Brenner, VMD,<sup>1\*</sup> Jonnie Capiro,<sup>2</sup> Catherine M. Terry,<sup>3</sup> and Lauren L. Howard, DVM, Dipl ACZM<sup>2</sup>*

<sup>1</sup>*Indianapolis Zoo, Indianapolis, IN 46222 USA;* <sup>2</sup>*San Diego Zoo Safari Park, Escondido, CA 92027 USA;* <sup>3</sup>*University of California Davis School of Veterinary Medicine, Davis, CA 95616 USA*

### Abstract

Wild rhinoceros populations are currently threatened due to poaching and habitat loss and captive populations are not self-sustaining.<sup>5,11,12</sup> Therefore, efforts towards maximizing captive breeding efforts are critically important. While there have been great increases in knowledge of rhinoceros reproductive physiology and breeding management, there is still a lack of understanding regarding what are considered normal parameters during parturition.<sup>7,9,10</sup> We reviewed data regarding parturition of two rhinoceros species (southern white rhinoceros [SWR], and greater one-horned rhinoceros [GOH]) from videos, medical records, and literature of documented birthing events.<sup>1,4,8</sup> Using equine parturition parameters as a model for comparison, we compiled the following data on two species of rhinoceros: signs of impending parturition, duration of the three phases of parturition, and normal calving presentation.<sup>2,3,6</sup> Preliminary data from 11 animals (7 SWR, 4 GOH) and 16 births comparing calf presentation and viability documented 5 still births (4 posterior and 1 unknown presentation) and 11 live births (6 anterior, 1 posterior, and 4 unknown presentations). Ongoing data collection will lead to a more robust data set and will strive to include black rhinoceros (*Diceros bicornis*). The authors would like to stress the importance of investing in the monitoring of parturition, as detailed documentation is a necessary tool in determining normal parameters. The data presented in this review are intended to aid facilities with rhinoceros breeding programs and to provide prospective standardization of parturition observation parameters.

**Key words:** Calf presentation, parturition phases, pregnancy, rhinoceros, signs of impending parturition, stillborn calf

### ACKNOWLEDGMENTS

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## EVALUATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR PREGNANCY DETECTION IN OKAPI (*Okapia johnstoni*) AND GERENUK (*Litocranius walleri*)

Zachary T. Dvornicky-Raymond,<sup>1\*</sup> Kyle A. Donnelly, DVM, MPH,<sup>2</sup> Jessica A. Emerson, DVM, Dipl ACZM,<sup>2</sup> Linda M. Penfold, PhD,<sup>3</sup> and Scott B. Citino, DVM, Dipl ACZM<sup>2</sup>

<sup>1</sup>Cornell University College of Veterinary Medicine, Ithaca, NY 14850 USA; <sup>2</sup>White Oak Conservation, Yulee, FL 32097 USA; <sup>3</sup>South East Zoo Alliance for Reproduction & Conservation (SEZARC), Yulee, FL 32097 USA

### Abstract

Accurate and timely pregnancy diagnosis is crucial for population management of captive and wild ruminants. Pregnancy-associated glycoproteins (PAGs) are a large family of glycoproteins that are conserved across species and have been detected in domestic and wild bovids, ovids, caprids, and cervids.<sup>1-8</sup> PAGs are produced by placental trophoblasts and can reach detectable levels in maternal blood. This study evaluated the use of a commercially available, visual enzyme-linked immunosorbent assay (ELISA; IDEXX Rapid Visual Pregnancy Test, IDEXX Laboratories, Inc. Westbrook, ME 04092) for diagnosis of pregnancy in okapi (*Okapia johnstoni*) and gerenuk (*Litocranius walleri*). This assay has been validated for use in domestic cattle, sheep, goats, and water buffalo. Unlike other blood-based PAG detection methods, this assay does not require sophisticated laboratory equipment for detection or interpretation, and can therefore be utilized in many settings. Banked serum samples from gerenuk ( $n = 11$ ) and okapi ( $n = 3$ ) were tested, and a pregnant and non-pregnant sample from each individual were included. The ELISA showed 100% sensitivity and specificity in the gerenuk samples, and 0% sensitivity in okapi. In gerenuk, the earliest detected pregnancy was at approximately 7-8 wk gestation. These results are consistent with previous studies that were able to accurately detect pregnancy in other members of the family Bovidae,<sup>2,6</sup> but it is possible that PAGs present in okapi are too structurally dissimilar for detection using this test. This ELISA appears to be an accurate, rapid, and inexpensive method of point-of-care pregnancy diagnosis in gerenuk, but not okapi. Additional studies should be pursued to investigate the validity of this test in other non-domestic ruminant species.

**Key words:** Gerenuk, *Litocranius walleri*, okapi, *Okapia johnstoni*, pregnancy, pregnancy-associated glycoproteins

### ACKNOWLEDGMENTS

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## ULTRASONOGRAPHIC FINDINGS ASSOCIATED WITH NORMAL PREGNANCY AND FETAL WELL-BEING IN THE BOTTLENOSE DOLPHIN (*Tursiops truncatus*)

*Marina Ivančić, DVM, Dipl ACVR,<sup>1\*</sup> Forrest M. Gomez, DVM,<sup>2</sup> Whitney B. Musser, MSc,<sup>2</sup> Ashley Barratclough, BVetMed, MSc WAH, MS, MRCVS,<sup>2</sup> Jennifer M. Meegan, DVM, Dipl ACZM,<sup>2\*</sup> Sophie M. Waitt,<sup>3</sup> Abraham Cárdenas Llerenas, MVZ,<sup>4</sup> Eric D. Jensen, DVM,<sup>5</sup> and Cynthia R. Smith, DVM<sup>2</sup>*

<sup>1</sup>Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513 USA; <sup>2</sup>National Marine Mammal Foundation, San Diego, CA 92101 USA; <sup>3</sup>University of Southern California, Los Angeles, CA 90089 USA; <sup>4</sup>Dolphin Adventure, Nuevo Vallarta, Mexico; <sup>5</sup>U.S. Navy Marine Mammal Program, San Diego, CA 92152 USA

### Abstract

Reproductive success is vital in sustaining free-ranging bottlenose dolphin (*Tursiops truncatus*) populations and is a critical component of managed care. Ultrasonography is invaluable in wild dolphin health assessments and marine mammal preventive medicine. It is also a non-invasive standard of care in assessing the fetomaternal unit in humans and animals, including dolphins and horses. The purpose of this prospective longitudinal study was to develop a protocol for fetomaternal ultrasonographic monitoring in *Tursiops truncatus* and to report normal measurements and descriptive findings correlated with a positive outcome. From 2010-2017, serial ultrasonographic evaluations of 12 healthy dolphins in managed care were performed over the course of 16 pregnancies. A total of 203 ultrasound exams was included in the study. Several metrics were practical to obtain and accurate in predicting fetal age. Fetal biparietal diameter (BPD), thoracic width in dorsal and transverse planes, thoracic height in a sagittal plane, aortic diameter, and blubber thickness all demonstrated a high correlation with date of gestation ( $r > 0.94$ ,  $P < 0.00001$ ). Regional uteroplacental thickness significantly increased with each trimester (range 0.22-0.40 cm;  $P < 0.00011$  cranial uterus,  $P < 0.00057$  mid, and  $P < 0.000011$  caudal). Lung:liver mean pixel intensity was  $2.57 \pm 0.46$  (95% CI 2.47-2.67). Ultrasonographic characteristics of normal pregnancy in dolphins are described and an updated equation for prediction of parturition date in *Tursiops* is reported: days to parturition =  $348.16 - (26.03 * \text{BPD}[\text{cm}])$  ( $R^2 = 0.99$ ). Future applications of these normal data will help identify in utero abnormalities indicative of fetal distress and morbidity, and further improve understanding of reproductive failure in wild and managed populations.

**Key words:** Bottlenose dolphin, gestation, pregnancy, *Tursiops truncatus*, ultrasonography, ultrasound

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## A REVIEW OF THE CURRENT USE OF GONADOTROPIN-RELEASING HORMONE VACCINES FOR REPRODUCTIVE MANAGEMENT OF EUROPEAN AND NORTH AMERICAN ZOO ANIMALS

*Taina B. Strike, BVSc, MSc, DipECZM (Zoo Health Management), MRCVS,<sup>1,2\*</sup> Yedra Feltrer Rambaud, DVM, MSc, DipECZM (Zoo Health Management), MRCVS,<sup>2</sup> Veronica B. Cowl, PhD,<sup>2</sup> and Ashley Franklin, PhD<sup>3</sup>*

<sup>1</sup>*Zoological Society of London (ZSL), London Zoo, UK;* <sup>2</sup>*European Association of Zoos and Aquariums (EAZA) Group on Zoo Animal Contraception (EGZAC), Chester Zoo, UK;*

<sup>3</sup>*Association of Zoos and Aquariums (AZA) Reproductive Management Center (RMC), Saint Louis Zoo, St. Louis, MO 63110 USA*

### Abstract

The use of gonadotropin-releasing hormone (GnRH) vaccines in free-living wildlife is well documented. There is an increasing trend of their use in zoo animals given the relative ease of delivery of these products; however, there are few published reports of GnRH vaccine efficacy in zoo species and whether successes and failures are as a result of dosage, frequency of booster vaccination, or due to variation in species physiology. Reversibility of contraception and a return to full fertility after GnRH vaccination for individuals in captive breeding programs is crucial, but mostly unknown. Using the Association of Zoos and Aquariums (AZA) Reproductive Management Center (RMC) and European Association of Zoos and Aquaria (EAZA) Group on Zoo Animal Contraception (EGZAC) Contraception Database, we reviewed the use of GnRH vaccines (Improvac<sup>®</sup>/Improvest<sup>®</sup>, GnRH conjugate vaccine, Zoetis<sup>®</sup>, Parsippany, NJ 07054 USA) in European and North American zoos over the last 10 yr. In total, 686 bouts of vaccination were recorded in 148 (56:92) individuals, comprising 47 different species. Vaccines were principally used in ungulates (88% of records), but were also used in elephants (9%), pinnipeds (1.3%), rodents (0.6%), felids (0.3%), and macropods (0.2%). Vaccines were primarily used for contraception of male (121 records) and female (386 records) animals, but also for aggression management (82 records) and for the therapeutic treatment of reproductive tract disorders (97 records). Full contraceptive reversal was documented with live births in three out of nine animals that were allowed to breed; however, several more conceived due to contraception failure or incorrect product use. The review has helped identify successful contraception as well as causes of failure and our current understanding of how best to use GnRH vaccines in different zoo species.

**Key words:** Contraception, GnRH, gonadotropin-releasing hormone, ungulates, vaccine

### ACKNOWLEDGMENTS

The authors thank the institutions that have entered their data on the EAZA Group on Zoo Animal Contraception and the AZA Reproductive Management Centre websites, information that has anonymously contributed to this study.

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## THE SUSTAINABILITY CRISIS IN ZOOS NEEDS BOTH ARCHIVAL AND PROSPECTIVE STUDIES: THE REPRODUCTIVE HEALTH SURVEILLANCE PROGRAM HAS BOTH

*Dalen W. Agnew, DVM, PhD, DACVP<sup>1,2\*</sup> and Anneke Moresco, DVM, PhD<sup>1,3</sup>*

*<sup>1</sup>Reproductive Health Surveillance Program, <sup>2</sup>Michigan State University, Lansing, MI 48824 USA; <sup>3</sup>Denver Zoo, Denver CO 80205 USA*

### Abstract

The Reproductive Health Surveillance Program (RHSP) has been archiving reproductive tissues from a large variety of species, ages, and contraceptive treatments since 1995 and currently holds over 3,000 cases.<sup>3</sup> This archive allows for valuable retrospective research that has identified important trends in reproductive disease such as the long-term carcinogenic effects of MGA in felids,<sup>5</sup> or more recently, that female suids develop a range of endometrial lesions ranging from hyperplasia to malignant cancers, but that there was no correlation of uterine lesions and contraceptive use,<sup>2</sup> or that zebras do not develop significant ovarian or endometrial lesions after administration of PZP. Although very valuable, archived samples will never have the consistency in collection methods that a prospective study can have. When the RMC is contacted about clinical cases, the RHSP actively participates in advising, using the information gained through the archive. Therefore, the RHSP also currently supports more prospective studies. A few examples include: 1) We are evaluating the genetic factors for the remarkable propensity of African painted dogs (*Lycaon pictus*) to develop adenomyosis and pyometra. 2) Carnivores (felids in particular) seem to have long reversibility intervals after treatment with deslorelin;<sup>4</sup> however, it is unknown if this latency period is due to very low levels of deslorelin release from the implant or due to a strong suppression of the pituitary receptors. A new assay is being developed to measure deslorelin in serum at pg/ml concentrations. The next step will be to use clinical samples to better characterize serum levels and tailor administration to each species. 3) Evaluation of the efficacy of Improvest® (GnRH conjugate vaccine, Zoetis®, Parsippany, NJ 07054 USA) a new contraceptive method in giraffe (*Giraffa camelopardalis*), is currently underway by serial collection of non-invasive samples and eventually archiving tissues from necropsy. 4) Transcervical biopsy, already validated in domestic dogs (*Canis familiaris*), is being assessed prospectively as a tool to evaluate early endometrial hyperplasia in non-domestic canids.<sup>1</sup> As the zoo community works together to address the crisis in sustainability, it is critical to support both prospective studies and the responsible archival of biological specimens which will provide valuable insight into our continued efforts to manage reproduction.

**Key words:** Archival studies, contraception, prospective studies, reproductive pathology, sustainability

### ACKNOWLEDGMENTS

The AZA Reproductive Management Center and its advisory board have been and continue to be a valued collaborator in all these studies. We also appreciate the great support from zoos, veterinarians, and pathologists have provided for these studies and the continued additions to the RHSP archive.

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## CARE, HUSBANDRY, AND USE OF FREE ONLINE INFORMATION

*Heather K. Moberly, MSLS, AHIP, FHEA, Pg Cert (Veterinary Education)*

*Texas A&M University, College Station, TX 77843 USA*

### Abstract

This talk provides tips, techniques, and resources that you can put into use immediately to search for and find veterinary information online. It is about searching to discover what has been published about a topic; finding newer articles that reference an article you already have; and downloading copies of full-text articles. All resources discussed are available online and, with one notable exception, all are free.

**Key words:** Free, information, internet, online, searching, veterinary

### INTRODUCTION

The care and husbandry of information is most often a collaboration among authors, publishers, and librarians. Certainly individuals and organizations may contribute to more than one role in the lifecycle of a work, but a simplified view suffices here: Authors have ideas, gather information, and create works. Publishers guide refinement, format structure, and distribute works. Librarians select and collect works, create ways to locate information, and house works for posterity. Our topic, free online information, has a lot of awkward moments. This is one of them. All these steps require effort and payment; there is no such thing as a free lunch. Or, to use a veterinary metaphor, there is no such thing as a free kitten. Keep that in the back of your mind for later and let's move on.

In the context of this talk, free means using or acquiring something at no financial cost to you at the time you use it. It means the true cost of, for example a free online copy of an article, has been paid at a different time and through a different method than you paying to read or download it here and now. This takes a number of forms and is described with a variety of phrases but Open Access is a good phrase to know. Open Access, or OA, resources and publications mean that the distribution of, and access to, the item is without cost to the end recipient. That OA refers to distribution bears repeating. The ownership or copyright of the intellectual content is different than distribution. OA does not mean the intellectual content is in the public domain or copyright free.

To keep formatting simple, the URLs for all resources are listed at the end of this paper.

### WHY ARE WE SEARCHING?

To begin, why are we talking about finding published information to practice veterinary medicine? One reason is that identifying and acquiring published evidence are integral steps in the Evidence-Based Veterinary Medicine methodology. To learn more try the peer-reviewed, interactive tutorial, EBVM Learning. A new edition is expected in early 2020.

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## SEARCHING

I'm often asked how to create the best single search the first time. The answer is that successful searching is iterative.

Searching is most successful when you have deliberately thought about what it is you need to find. It is both an art and a science. The art is the creativity and subject knowledge needed to decide what you're looking for, whether it is likely to have been published, where it would have been published, and how you can locate it. The science is learning how online searches work and leveraging that knowledge. You do that by carefully choosing search terms, combining those terms with Boolean Algebra operators (AND, OR, NOT), and searching both forward and backward in the literature (looking at cited references and citing articles). The EBVM tutorial mentioned above can help as can the peer-reviewed PubMed for Veterinarians tutorial.

There is no single, all-encompassing index to articles that covers veterinary literature, zoo or otherwise. The best depends, of course, on what you are looking for in each search. PubMed is an index to articles published in the biomedical sciences. It covers a good hundred or so journals that comprise a core of veterinary medical literature. Interdisciplinary coverage crossing into the biomedical sciences can be a strength when searching for veterinary topics. The search interface is relatively intuitive; online help and tutorials are available. Results include several useful additional links: Similar Articles and Full Text Links. PubMed can be customized with a My NCBI account. IVIS is a resource with a particular strength in searchable full-text veterinary proceedings; it requires you to create an account. Although this is a talk about free resources, I would be remiss if I did not mention VetMed Resource. It requires a paid subscription and includes CAB Abstracts, the most comprehensive veterinary literature index.

Looking for research syntheses can be a great strategy. There are not a lot of these in veterinary medicine but the number is growing. Systematic Reviews are secondary literature that investigate a specific question by identifying, appraising, and summarizing published research instead of conducting an original study. VetSRev is a database of citations for veterinary systematic reviews. Less extensive syntheses are also available. Two good resources for these are Best Bets for Vets and Knowledge Summaries published in the journal Veterinary Evidence. Each of these publishes the question, search strategy, resources evaluated, and a bottom line answer. An additional use of syntheses is consulting their strategies for search ideas.

## FINDING FREE FULL TEXT ARTICLES

When you seek the full-text of a specific article, there are several excellent tools: Unpaywall and OA Button. Unpaywall is a plug-in for your internet browser. Plug-ins, also called a browser extensions, are executable programs. OA Button has both a plug-in and a searchable website. If you do not want to install plug-ins, you can visit the OA Button website to search for the full text of an article.

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## GOOGLE SCHOLAR

Google Scholar receives a heading because it is a multi-faceted tool. It is a better alternative when searching for articles than Google because it searches only for articles. It has a plug-in which can be efficient to want to pop in and use it quickly from a different webpage.

In addition to the tools above, Google Scholar is an excellent tool to locate free full text articles. Search Google Scholar for the article title in quotation marks, “ ”, for the best results.

Yes, searching Google Scholar for your topic can identify articles not found in other searches. Google sees different online information than other resources and uses a proprietary search algorithm. Remember that Google search results can be impossible to replicate so capture results you want when you find them. The nature of Google means they change their algorithm at will and the nature of the Internet means Google doesn't always see the same content.

Along the bottom of each result is a line of links that are worth exploring. The All Versions link will show all the locations Google found for that single article. Sometimes this includes additional full article links which are useful if one doesn't work.

Google Scholar results include two quick ways to expand your search results without creating a new search. The cited by link displays a list of articles Google located that cite the article you are viewing. The related articles link locates similar articles.

## THE BOTTOM LINE

Is there a free lunch? No. There is always a Caveat Emptor with free resources. A question to ask yourself is, if you are not paying for the resource or product, who is? Are you the product? If you are not paying cash at a paywall, are you creating a login and paying through a datawall? For some resources the cost is tracking your behavior. For others, you receive targeted advertising. It is a fast moving model and increasingly intrusive to privacy. The bottom line is that you need to make choices you are comfortable with. Do a bit of research. Be aware. Be smart online.

Can you locate and acquire everything you need to practice veterinary medicine online and free? It is unlikely enough that we can confidently say no. Using freely available resources can help you stretch and allocate available funds in the best way for your information needs.

## RESOURCES IN THE ORDER PRESENTED

EBVM Learning tutorial

<https://ebvmlearning.org>

PubMed for Veterinarians tutorial

[http://cases.vetmoodle.org/CET\\_CoursePlayer/demo1/public/pubmed.html](http://cases.vetmoodle.org/CET_CoursePlayer/demo1/public/pubmed.html)

PubMed

<https://www.ncbi.nlm.nih.gov/pubmed>

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IVIS

<https://www.ivis.org>

VetMed Resource

<https://www.cabi.org/vetmedresource>

VetSRev

<http://webapps.nottingham.ac.uk/refbase>

Best Bets for Vets

<https://bestbetsforvets.org>

Veterinary Evidence

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Unpaywall

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Google Scholar

<https://scholar.google.com>

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## A RETROSPECTIVE ANALYSIS OF AMOEBIASIS IN REPTILES IN A ZOOLOGICAL INSTITUTION

Alexander JD. McFarland, BA,<sup>1\*</sup> Kenneth J. Conley, DVM, DACVP,<sup>2</sup> Tracie A. Seimon, PhD,<sup>2</sup> and John M. Sykes IV, DVM, Dipl ACZM<sup>2</sup>

<sup>1</sup>The Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Science, Colorado State University, Fort Collins, CO 80523 USA; <sup>2</sup>Wildlife Conservation Society, Zoological Health Program, Bronx, NY 10460 USA

### Abstract

Amoebiasis is a significant protozoal disease of reptiles. It is thought to be caused by *Entamoeba invadens* carried asymptotically by chelonians and then transmitted to squamates in whom disease then develops; however, this paradigm is not always seen. Investigating the pathophysiology of amoebiasis has been hampered by the inability to speciate amoeba using conventional techniques. This study reviewed necropsy records from WCS collections from 1998-2017. Amoeba were identified histologically in 54 cases. Of these, amoeba were the cause of death in 32 (18 chelonians, 7 lizards, and 7 snakes), a significant co-morbidity in 14 (6 chelonians, 2 lizards, and 6 snakes), and seen incidentally in 8 cases (1 chelonian, 6 lizards, and 1 snake). 65% of cases had been moved within 180 days of death (median 46 days). Frozen tissue samples from 19 of these cases were tested via an *Entamoeba* (genus-specific) PCR assay. PCR products were sequenced and speciated. Six individuals were positive for *E. invadens* (three chelonians, two snakes, one lizard), two for *E. ranarum* (both snakes), and one for *E. terrapinae* (chelonian). Amoebiasis was the cause of death or a co-morbidity in nine of the 10 PCR-negative cases, possibly due to using primers specific to *Entamoeba* genus. *E. ranarum* has typically been thought to be a disease of amphibians and has only been reported once to cause disease in a snake. *E. terrapinae* has only been reported incidentally in chelonians. These results suggest that amoebiasis is a complicated and nuanced disease of reptiles, and warrants additional study.

**Key words:** Amoebiasis, *Entamoeba invadens*, *Entamoeba ranarum*, *Entamoeba terrapinae*

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## DISENTANGLING THE EFFECTS OF SEASON AND TEMPERATURE ON HEMATOLOGIC VALUES IN PRAIRIE RATTLESNAKES (*Crotalus viridis*)

*Matthew C. Allender, DVM, MS, PhD, Dipl ACZM,<sup>1,2\*</sup> Ethan Kessler, BS,<sup>2</sup> Sarah J. Baker, PhD,<sup>3</sup> Ellen Haynes, DVM,<sup>1</sup> and Amy Schnelle, DVM, MS, DACVCP<sup>4</sup>*

*<sup>1</sup>Wildlife Epidemiology Laboratory, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 USA; <sup>2</sup>Illinois Natural History Survey, Prairie Research Institute, University of Illinois, Champaign, IL 61802 USA; <sup>3</sup>Arizona Department of Fish and Game, Phoenix, AZ 85086 USA; <sup>4</sup>Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL 61802 USA*

### Abstract

Hematologic assessment is the most common clinical tool used by practitioners and researchers to characterize both individual and population health. In reptiles, the hematologic response can be influenced by factors such as temperature and season, especially in temperate species. To improve the diagnostic utility of hematology in reptiles, it is imperative to evaluate and characterize the normal range of physiologic variation. The study objectives were to 1) determine the impact of temperature and time of year on CBC parameters; and 2) create subject-based reference intervals for 20 prairie rattlesnakes (*Crotalus viridis*). Animals were randomly assigned to either a control group housed at a constant temperature (75-77 °F) or a treatment group housed in an environmental chamber with the temperature altered to reflect natural seasonal variation in temperature. Twice monthly for 1 yr, blood samples were collected and the following hematologic parameters were measured: total white blood cell count (WBC), packed cell volume (PCV), total solids (TS), and white blood cell differential counts. WBC decreased and PCV increased as the mean previous 14-day temperature increased, with no effect of season. Total solids were higher in the control group, but there was no direct effect of temperature or season. These results suggest that environmental temperature, rather than season, drives hematologic parameters, which should prove useful when interpreting hematologic assessment results of reptiles.

**Key words:** *Crotalus viridis*, hematology, reference intervals

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## SALMONELLOSIS IN STRANDED AND PELAGIC OLIVE RIDLEY TURTLES (*Lepidochelys olivacea*) IN THE PACIFIC

*Terry M. Norton, DVM, Dipl ACZM,<sup>1,2\*</sup> Thierry M. Work,<sup>3</sup> Julie Dagenais,<sup>3</sup> Brian A. Stacy,<sup>4</sup> Jason T. Ladner,<sup>5</sup> Jeffrey M. Lorch,<sup>6</sup> Thomas Waltzek,<sup>7</sup> George H. Balazs,<sup>8</sup> Brenda M. Berlowski-Zier,<sup>6</sup> Renee Breeden,<sup>3</sup> Elías Barquero-Calvo,<sup>9</sup> Isabel Contreras,<sup>10</sup> Natalia Corrales Gomez,<sup>11</sup> Rocío González- Barrientos,<sup>12</sup> Heather S. Harris,<sup>13</sup> Ángel Herrera-Ulloa,<sup>11</sup> Gabriela Hernández-Mora,<sup>14</sup> Shoreh Hesami,<sup>15</sup> T. Todd Jones,<sup>6</sup> Juan Alberto Morales,<sup>9</sup> Carlos Mario Orrego,<sup>15</sup> Robert A. Rameyer,<sup>3</sup> and Daniel R. Taylor<sup>6</sup>*

<sup>1</sup>Georgia Sea Turtle Center, Jekyll Island Authority, Jekyll Island, GA 31527 USA; <sup>2</sup>Turtle Hospital, Marathon, FL 33050 USA; <sup>3</sup>US Geological Survey, National Wildlife Health Center, Honolulu Field Station, Honolulu, HI 96850 USA; <sup>4</sup>NOAA Fisheries, Office of Protected Resources, University of Florida, Gainesville, FL 32611 USA; <sup>5</sup>The Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ 86011 USA; <sup>6</sup>US Geological Survey, National Wildlife Health Center, Madison, WI 53711 USA; <sup>7</sup>Department of Infectious Diseases & Immunology, University of Florida, Gainesville, FL 32611 USA; <sup>8</sup>NOAA, National Marine Fisheries Service, Marine Turtle Program, Honolulu, HI 96822 USA; <sup>9</sup>Escuela de Medicina Veterinaria (EMV), Universidad Nacional Costa Rica, Costa Rica; <sup>10</sup>Fundacion Zoologica de El Salvador, Antiguo Cuscatlán, La Libertad, El Salvador; <sup>11</sup>Parque Marino del Pacífico-Universidad Nacional Costa Rica, Costa Rica; <sup>12</sup>Pathology Area National Service of Animal Health (SENASA), Ministry of Agriculture and Livestock, Costa Rica, California Department of Fish and Wildlife, Office of Spill Prevention and Response; <sup>13</sup>Marine Wildlife Veterinary Care and Research Center, Santa Cruz, CA USA; <sup>14</sup>Bacteriology Area, National Service of Animal Health (SENASA), Ministry of Agriculture and Livestock, Costa Rica; <sup>15</sup>Costa Rican Ministry of Environment and Energy, San Jose, Costa Rica

### Abstract

*Salmonella* spp. are frequently shed by wildlife including turtles, but *S. enterica* subsp. *enterica* serovar Typhimurium is rare in the latter. Moreover, the presence of lesions associated with *Salmonella* is unusual in chelonians. Using necropsy, immunohistochemistry, PCR, and culture, we showed that 11% of apparently healthy pelagic and 57% of stranded olive ridley turtles (*Lepidochelys olivacea*) on the Pacific coast of Central America, the continental United States, and Hawaii had renal granulomas associated with *S. enterica* ser. Typhimurium. The extent of renal lesions ranged from mild, focal granulomas to severe nephritis. Some animals were found during mass mortality events in Central America, suggesting that salmonellosis may have been a contributing cause. *S. enterica* ser. Typhimurium was the only species of *Salmonella* detected in olive ridley turtles, and phylogenetic analyses from whole-genome sequencing data showed that the isolates from olive ridley turtles formed a single clade distinct from other strains of *S. enterica* ser. Typhimurium. Although *S. enterica* ser. Typhimurium is known in coastal environments, its presence in olive ridley turtles within the oceanic environment is unexpected. Further study is warranted to determine the origin and host specificity of the olive ridley turtles *S. enterica* ser. Typhimurium strain as well as the potential role of salmonellosis in olive ridley turtle conservation. In addition, *S. enterica* ser. Typhimurium is one of the more common non-typhoidal *Salmonella*

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in humans, and olive ridley eggs are commonly harvested for human consumption in Central America, thus a potential for human exposure exists.

**Key words:** Olive ridley turtle, *Salmonella*, *Salmonella enterica*, *Salmonella enterica* ser. Typhimurium

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## CHARACTERIZING THE EPIDEMIOLOGY OF HISTORIC AND NOVEL PATHOGENS IN BLANDING'S TURTLES (*Emydoidea blandingii*)

*John M. Winter, BS,<sup>1\*</sup> Matthew C. Allender, DVM, MS, PhD, Dipl ACZM,<sup>1</sup> Lauren Mumm, BS,<sup>1</sup> Laura A. Adamovicz, DVM, PhD,<sup>1</sup> Kirsten E. Andersson, BS,<sup>1</sup> and Gary A. Glowacki, BS, MS<sup>2</sup>*

<sup>1</sup>*University of Illinois Wildlife Epidemiology Laboratory, College of Veterinary Medicine, Urbana, IL 61802 USA;* <sup>2</sup>*Lake County Forest Preserve District, Libertyville, IL 60048 USA*

### Abstract

Pathogens such as herpesviruses, *Mycoplasma* spp., and frog virus 3-like ranavirus have contributed to morbidity and mortality in many species of free-living and zoo-maintained chelonians; however, their prevalence is understudied in Blanding's turtles (*Emydoidea blandingii*) across North America. To assess presence of these pathogens, Blanding's turtles were sampled in Lake County, Illinois in 2017 ( $n = 213$ ) and 2018 ( $n = 160$ ). DNA from cloacal-oral swabs was assayed for four ranaviruses, three *Mycoplasma* spp. two *Salmonella* spp., *Emydoidea* herpesvirus 1 (EBHV1), and tortoise intranuclear coccidiosis using a multiplex quantitative polymerase chain reaction (qPCR). Pathogens were most frequently detected in adult turtles ( $n = 25$ ), and rarely in subadults ( $n = 2$ ) or juveniles ( $n = 1$ ). EBHV1 was detected in 22 individuals with few clinical signs of illness, most ( $n = 20$ ) occurring in the month of May ( $P < 0.0001$ ). EBHV1 positive turtles had lower relative lymphocyte counts and higher relative basophil counts and heterophil:lymphocyte ratios ( $P < 0.05$ ). EBHV1 cases at one study site significantly clustered within the same 0.64 km area from May 17 through May 22, 2017 ( $P < 0.0001$ ) and May 14 through May 15, 2018 ( $P = 0.0006$ ). Individuals were rarely positive for *Salmonella enterica* ser. Typhimurium ( $n = 6$ ). A novel *Mycoplasma* sp. sharing high homology with other emydid *Mycoplasma* spp. was detected in one turtle with nasal discharge using conventional PCR. Continued monitoring of this population and habitat may facilitate identification of risk factors for pathogen occurrence and clarify the impact of infectious diseases on Blanding's turtle conservation outcomes.

**Key words:** *Emydoidea blandingii*, herpesvirus, *Mycoplasma* spp., ranavirus, *Salmonella enterica* ser. Typhimurium

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## THE EFFECT OF VARIED ENRICHMENT ON SNAKE BEHAVIOR

*Sneha Krishnan, BS,<sup>1\*</sup> Miranda Sadar, DVM, Dipl ACZM,<sup>1</sup> Sangeeta Rao, BVSc, MVSc, PhD,<sup>1</sup> and Eric Klaphake, DVM, Dipl ACZM, DABVP (Avian Practice)<sup>2</sup>*

*<sup>1</sup>Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO 80523 USA; <sup>2</sup>Cheyenne Mountain Zoo, Colorado Springs, CO 80906 USA*

### Abstract

Environmental enrichment is a strategy used to improve the welfare of captive animals.<sup>1</sup> While enrichment techniques for mammals and birds have been studied extensively, reptilian enrichment has received less attention.<sup>2</sup> There has been an increase in enrichment programs for reptiles in zoos, however many are not accompanied by behavioral studies. Detailed recording of behavioral responses to enrichment is necessary to assess the efficacy of the enrichment type, and to determine its utility in various settings.<sup>2</sup> In this study, 18 snakes of varying species (Colubridae, Pythonidae) were exposed to four enrichment types (humid hide, olfactory, climbing, suspended hide). Baseline recordings were conducted prior to the introduction of enrichment. Snakes were recorded for 2 hr after introduction of each item. Five behavior types were identified based on evaluation of baseline videos: tongue flicking, climbing, hiding, glass climbing, and utilizing other items. Glass climbing was classified as a negative behavior while the other four behaviors were classified as positive.<sup>3</sup> An increase in climbing and tongue flicking was seen with introduction of each enrichment item. The increase in these behaviors may indicate clinical importance. A trend towards significance was seen in the reduction of glass climbing behavior after the introduction of enrichment. These results show that captive snakes respond to environmental enrichment. The reduction in negative behavior frequency may suggest increased welfare. The extent to which results can be applied to other species awaits further study, but the authors suggest that climbing and olfactory enrichment be utilized to improve snake welfare.

**Key words:** Colubridae, environmental enrichment, olfactory enrichment, Pythonidae, snake behavior

### ACKNOWLEDGMENTS

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## INSIGHT INTO CHELONIAN INNATE IMMUNE RESPONSE BY TRACKING CYTOKINE TRANSCRIPTION OVER TIME IN EXPERIMENTAL AND FREE-RANGING POPULATIONS

Jeremy M. Rayl, PhD,<sup>1\*</sup> Matthew C. Allender, DVM, MS, PhD, Dipl ACZM,<sup>1</sup> Laura Adamovicz, DVM, PhD,<sup>1</sup> and Christopher A. Phillips, PhD<sup>2</sup>

<sup>1</sup>Wildlife Epidemiology Laboratory, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 USA; <sup>2</sup>Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign, Champaign, IL 61820 USA

### Abstract

Global reptile populations face numerous threats to viability, including disease.<sup>3</sup> In chelonians and other herptile species, innate immunity is an important factor in host response to infectious disease.<sup>5</sup> Cytokines are transient signaling molecules that trigger a myriad of responses in the vertebrate innate immune system.<sup>1</sup> Cytokine mRNA transcription analysis has been used in other vertebrates as a measure of the innate immune response.<sup>2,4</sup> To evaluate the chelonian immune response, we designed assays for three cytokine targets in chelonians for experimental and field testing; interleukin 1-beta (IL1B) and tumor necrosis factor-alpha (TNF) which are pro-inflammatory, and interleukin 10 (IL10), which is anti-inflammatory. As a model species, red-eared sliders (*Trachemys scripta elegans*) were challenged with frog virus-3 at two temperatures (28 °C, 22 °C) and samples were collected twice weekly over 30 days for hematology, pathogen detection, and cytokine mRNA transcription analysis. IL1B was significantly greater in infected turtles compared to uninfected turtles, with differences in transcription across time frames and temperatures. TNF and IL10 were transcribed at detectable levels, but were not significantly different between groups. In free-ranging eastern box turtles, samples were collected bi-weekly over two active seasons (2016-2017) for hematology, pathogen detection, and cytokine mRNA transcription analysis. IL1B transcription was highest in the summer of 2016 and the spring of 2017, while transcription for TNF and IL10 were similar between seasons and years. IL1B and IL10 transcription were greater in females. Using these assays, we seek to elaborate chelonian health by quantifying host response to pathogens and the environment.

**Key words:** Chelonian, cytokine, eastern box turtle, innate immunity, red-eared slider

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## ASSESSMENT OF MOJAVE DESERT TORTOISE HEALTH INSIDE AND OUTSIDE THE LARGE-SCALE TRANSLOCATION SITE

*Josephine Braun, DrMedVet,<sup>1\*</sup> Tristan Burgess, BVSc, PhD,<sup>2</sup> Carmel Witte, MS, PhD,<sup>1</sup> Nadine Lamberski, DVM, Dipl ACZM, DipECZM (Zoo Health Management),<sup>3</sup> Roy Averill-Murray, MS,<sup>4</sup> Kimberleigh Field, MS,<sup>4</sup> Linda Allison, MS,<sup>4</sup> and Bruce Rideout, DVM, PhD, DACVP<sup>1</sup>*

*<sup>1</sup>Disease Investigations, Institute for Conservation Research, San Diego Zoo Global, San Diego, CA 92112 USA; <sup>2</sup>Acadia Wildlife Services, South Freeport, ME 04078 USA; <sup>3</sup>Animal Health Department, San Diego Zoo Global, Escondido, CA 92027 USA; <sup>4</sup>Desert Tortoise Recovery Office, U.S. Fish and Wildlife Service, Reno, NV 89502 USA*

### Abstract

Desert tortoises (*Gopherus agassizii*) are impacted by continuous habitat loss and fragmentation. This study assesses health risks from improving connectivity between populations inside and outside the enclosed Large-scale Translocation Site (LSTS), Ivanpah Valley, Nevada. The LSTS was a recipient site for desert tortoises with diverse backgrounds. A total of 421 tortoises with health assessments in 2016 (T2) and a subset of 196 tortoises with prior health assessments between 2011 and 2014 (T1) were evaluated. The focus was on upper respiratory tract disease (URTD) caused by *Mycoplasma agassizii* and *M. testudineum*. Clinical signs were recorded at field health assessments. Infection status was determined by quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA). Multiple logistic regression was used to estimate associations while controlling for covariates. LSTS had the highest prevalence of *M. agassizii* (25%), *M. testudineum* (3.0%), and clinical signs (18.9%) in 2016, and the highest incidence of *M. agassizii* infection ( $P < 0.0001$ ). Presence of any clinical sign was positively associated with *M. agassizii* infection (OR=7.7,  $P < 0.0001$ ) but not with study site ( $P \geq 0.127$ ). There was no association with *M. testudineum* infection ( $P = 0.360$ ). An estimated 3.2% of tortoises converted from *M. agassizii*-negative to -positive between T1 and T2. This conversion was associated with increased prevalence of clinical signs at T2 (OR = 11.1,  $P = 0.018$ ). While *M. agassizii* and URTD are present inside and outside the LSTS, there is a possibility that incidence of *M. agassizii* infection and URTD would increase outside of LSTS if the two populations were to reconnect. The degree of risk and long-term population impact remain unknown.

**Key words:** Desert tortoise, ELISA, *Gopherus agassizii*, Large-scale Translocation Site, *Mycoplasma agassizii*, qPCR

### ACKNOWLEDGMENTS

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## LIVING SYRINGES: A PILOT STUDY USING HEMATOPHAGOUS TRIATOMINE INSECTS (*Triatoma dimidiata*) FOR BLOOD COLLECTION FROM ZOO REPTILES

Ellie L. Milnes, VetMB, DVSc,<sup>1,2,3\*</sup> Pauline Delnatte, DVM, DVSc, Dipl ACZM, DipECZM (Zoo Health Management),<sup>1</sup> Lydia Attard, MSc,<sup>1</sup> and Christopher Dutton, BSc, BVSc, MSc, Dipl ACZM, DipECZM (Zoo Health Management)<sup>1</sup>

<sup>1</sup>Toronto Zoo, Toronto, ON M1B 5K7, Canada; <sup>2</sup>Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada; <sup>3</sup>Current address: Ol Jogi Wildlife Conservancy, Nanyuki 10400, Kenya

### Abstract

Some species of triatomine insects, a subfamily of Reduviidae, feed on vertebrate blood by puncturing the host's skin with their proboscis.<sup>1,2</sup> The insect bite is painless, and the proboscis is much smaller than a hypodermic needle, producing minimal tissue trauma. Studies on mammals and birds have shown that biochemistry, hematology, and serology can be performed on insect-derived blood samples and generally yield results comparable to conventionally-collected samples.<sup>1,2</sup> The objective of this pilot study was to investigate the use of triatomine insects as living syringes to collect blood from zoo reptiles. A colony of *Triatoma dimidiata* (Belize kissing bugs) were acquired from the Centers for Disease Control (Atlanta, USA). Paired blood samples were collected from red-bellied short-necked turtles (*Emydura subglobosa*,  $n = 5$ ) by conventional venipuncture from the jugular vein versus blood collected by triatomines. Hematologic and biochemistry parameters were measured by an external laboratory. The quality of blood smears made from blood collected using both methods was comparable. Results showed statistically significant correlation between insect-derived and conventionally-collected blood parameters. Insect-derived samples had higher hematocrit, uric acid, amylase, aspartate aminotransferase, and globulin levels than jugular samples. Differences in insect-derived samples were likely due to hemolysis and digestive processes within the insect. The use of triatomine insects for blood sampling zoo animals may be useful for animals that would otherwise have to be anesthetized or strongly restrained for venipuncture, taxa that are difficult to bleed using conventional methods, and research protocols requiring an animal that is not stressed by human handling.

**Key words:** Blood sampling, *Emydura subglobosa*, hematophagy, reptile, *Triatoma dimidiata*, triatomine insect

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## ESTABLISHING TISSUE CULTURE CELL LINES FROM REPTILES AND AMPHIBIANS

*Robert J. Ossiboff, DVM, PhD, DACVP,<sup>1,2\*</sup> Tracey D. Logan,<sup>1</sup> and Tesla E. Richards<sup>2</sup>*

*<sup>1</sup>School of Natural Resources and Environment, University of Florida, Gainesville, FL 32611 USA; <sup>2</sup>Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 USA*

### Abstract

Tissue culture cell lines are a critical reagent for infectious disease research, comparative biomedical research, and can serve as a repository of genetic information for wildlife conservation. While a wide variety of cell lines are available from humans, domesticated and laboratory mammals, and even fish, cell lines from wildlife species, and particularly reptiles and amphibians, are extremely limited. As part of an ongoing project to increase the availability and access to reagents for wildlife disease research, we are establishing continuously dividing cell lines from reptile and amphibian species. Cell lines are derived from primary tissues collected at the time of necropsy, portions of surgical biopsies, or from embryonic tissues culled for management purposes. Primary cell growth was routinely observed from a variety of tissues, including heart, spleen, kidney, skeletal muscle, liver, and gonad. Continued selection for stable, dividing cells of homogenous morphology was achieved through passage in growth conditions determined optimal for the Class of the origin host. Aliquots of cell lines were routinely archived in liquid nitrogen to create low passage stocks. After 10-12 serial passages characterized by stable, predictable growth of cells representing a single cellular morphology, the cell line was considered established and aliquots of working stock cells were archived in liquid nitrogen. Final cell line characterization includes confirmation of host origin by sequencing of the cytochrome oxidase 1 gene, and immunohistochemical characterization to determine histogenesis when possible. At the time of submission, cell lines have been successfully established of various tissues from a variety of reptile species, including snakes ( $n = 6$ ), turtles/tortoises ( $n = 4$ ), crocodylians ( $n = 4$ ), and lizards ( $n = 2$ ). Additional cell lines from other species of reptiles and amphibians are currently in various stages of development. Cell lines will be cataloged in a publicly accessible virtual database to permit and promote the dissemination of these reagents to improve disease diagnostics and characterization of captive and free-ranging wildlife species.

**Key words:** Amphibian, cell culture, disease diagnostics, in vitro cell lines, reptile, virus isolation

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## FUNGAL SHELL DISEASE ASSOCIATED WITH *Emydomyces testavorans* IN A ZOO COLLECTION OF WESTERN POND TURTLES (*Actinemys marmorata*)

**Geoffrey R. Browning, MS, DVM,<sup>1,2\*</sup> Louden Wright, DVM,<sup>1,2,3</sup> Ray F. Wack, DVM, Dipl ACZM, DipECZM (Zoo Health Management),<sup>1,2,4</sup> and Jenessa L. Gjeltema, DVM, Dipl ACZM<sup>1,2,4</sup>**

<sup>1</sup>Department of Medicine and Epidemiology, University of California Davis School of Veterinary Medicine, Davis, CA 95616 USA; <sup>2</sup>Sacramento Zoo, Sacramento, CA 95822 USA; <sup>3</sup>San Diego Zoo Global, San Diego, CA 92101 USA; <sup>4</sup>Karen C. Drayer Wildlife Health Center, University of California Davis School of Veterinary Medicine, Davis, CA 95616 USA

### Abstract

A novel onygenalean fungus, *Emydomyces testavorans*, has recently been isolated from shell lesions of both free-ranging aquatic turtles and those under human care, including Western pond turtles (*Actinemys marmorata*) in conservation programs.<sup>1</sup> This case series describes the clinical presentation, diagnosis, and treatment of both early and later-stage lesions in a group of zoo-housed Western pond turtles. Thirteen 3-mo to 2-yr-old Western pond turtles presented for superficial carapacial lesions characterized by keratin disruption, including depigmentation, flaking, thinning, and less commonly full-thickness necrotic lesions extending into underlying bone. Fungal culture and polymerase chain reaction (PCR) with sequencing for *Emydomyces* species were positive. Other diagnostics included bacterial culture, histopathology, hematology, biochemistry, radiography, and computed tomography. Management of this disease involved debridement of lesions when indicated and topical treatment with betadine and terbinafine, which helped to resolve clinical signs after 3-16 mo of treatment. Following this, six approximately 3- to 17-yr-old adult Western pond turtles developed new lesions ranging from keratin depigmentation to deeper ulcerative lesions, which were positive for *Emydomyces testavorans* on fungal culture, PCR, and fungal sequencing. Clinical signs improved with a combination of topical therapy and debridement. The clinical findings, diagnostic, and therapeutic approach outlined in this case series can aid clinicians in recognition and diagnosis of subtle lesions in early stages of disease and the management of more severe cases associated with this fungus in aquatic turtles. Further research is necessary to determine optimal treatment protocols and potential risk factors for this disease.

**Key words:** *Actinemys marmorata*, *Emydomyces testavorans*, fungal shell disease, western pond turtle

### ACKNOWLEDGMENTS

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**INCUBATION, PIVOTAL TEMPERATURE OF SEX DETERMINATION, AND  
ENDOSCOPIC SEXING OF THE BURMESE BLACK GIANT TORTOISE (*Manouria  
emys phayrei*)**

***Paul M. Gibbons, DVM, MS, DABVP (Reptile and Amphibian Practice)<sup>1,2</sup>***

***<sup>1</sup>Turtle Conservancy, Ojai, CA 93023 USA; <sup>2</sup>Avian & Exotic Veterinary Care, Portland, OR  
97213 USA***

**Abstract**

The Burmese black giant tortoise (*Manouria emys phayrei*) is critically endangered and native to Southeast Asia. *Manouria* is the most primitive genus of extant tortoises, inhabiting wet, cool, montane forests, feeding on bamboo and mushrooms, and exhibiting nest-building and nest-guarding behaviors. Clutch size can exceed 50 eggs. Temperature-dependent sex determination has been previously reported. This study aims to specify incubation temperatures that produce each sex and to describe the endoscopic appearance of juvenile gonads. Eggs ( $n = 56$ ) were collected from the nest of a single dam in a breeding group in a private collection, positioned on a bed of vermiculite:water 2:1 w/w, covered with moistened, loose sphagnum moss, and divided into three groups incubated at different temperatures. Hatchlings were raised communally for 10 mo before endoscopic sexing. Tortoises were anesthetized and gonads were photographed during endoscopic examination via the prefemoral fossa. Standard error for parameters, pivotal temperature, and transitional range of temperatures were estimated using a Bayesian method with Monte-Carlo with a Markov chain and Metropolis-Hastings algorithm using weighted average temperature during the thermosensitive period. Thirteen eggs hatched from each group, higher temperatures produced more females, and incubation duration decreased with increasing temperature. The pivotal temperature (95% CI) was 28.87°C (27.58-30.06 °C), with a transitional range of temperatures (95% CI) from 27.98°C (25.33-29.36 °C) to 29.29°C (28.90-31.54°C). Testes were elongated, solid yellow, smooth-surfaced, with a uniform reticulated internal vascular pattern. Ovaries were elongated, colorless to pale yellow, with irregularly distributed regions of superficial granularity characterized by refractile ring structures.

**Key words:** Burmese black giant tortoise, endoscopy, incubation, *Manouria emys phayrei*, pivotal temperature, temperature-dependent sex determination

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**CHARACTERIZATION OF A NOVEL MESOMYCETozoEAN INFECTION  
(*Dermotheca* sp.) IN A STATE-ENDANGERED SALAMANDER (*Ambystoma platineum*)  
AND A CO-OCCURRING COMMON SPECIES (*Ambystoma texanum*)**

*Laura Adamovicz, DVM, PhD<sup>1\*</sup> Daniel B. Woodburn, DVM, DACVP,<sup>2</sup> Stephany Virrueta Herrera,<sup>3</sup> Kelsey Low, BS,<sup>3,4</sup> Andrew R. Kuhns, MS,<sup>4</sup> John A. Crawford, MS, PhD,<sup>5</sup> Chris A. Phillips, PhD,<sup>4</sup> and Matthew C. Allender, DVM, MS, PhD, Dipl ACZM<sup>1</sup>*

<sup>1</sup>*Wildlife Epidemiology Laboratory, University of Illinois College of Veterinary Medicine, Urbana, IL 61802 USA;* <sup>2</sup>*Zoological Pathology Program, University of Illinois College of Veterinary Medicine, Urbana, IL 61802 USA;* <sup>3</sup>*Program in Ecology, Evolution, and Conservation Biology, University of Illinois, Urbana, IL 61802 USA;* <sup>4</sup>*Illinois Natural History Survey, Prairie Research Institute, Champaign, IL 61820 USA;* <sup>5</sup>*National Great Rivers Research and Education Center, East Alton, IL 62024 USA*

**Abstract**

Global amphibian declines have been attributed to anthropogenic factors and diseases including ranavirus and chytridiomycosis.<sup>1</sup> Mesomycetozoan parasites (order Dermocystida) can cause direct mortality and thus may drive amphibian population declines, but their ecology and epidemiology remain understudied.<sup>2-6,8</sup> Concerningly, these parasites have recently emerged in North American caudates, and studies on natural infections are important to determine their impact on threatened species.<sup>6</sup> We investigated the prevalence, gross and histologic appearance, and molecular phylogeny of a novel dermocystid in the state-endangered silvery salamander (*Ambystoma platineum*) and the co-occurring, non-threatened small-mouthed salamander (*Ambystoma texanum*) from Illinois. Silvery salamander health assessments were performed at six ephemeral wetlands in February and March 2016-2018. Beginning in 2017, single to multiple 1-3 mm raised, white, round to dumbbell-shaped skin nodules were identified in 24 silvery salamanders and two small-mouthed salamanders from five wetlands (prevalence = 0-11.1%). Histologic evaluation of skin biopsies ( $n = 3$ ) was consistent with dermocystid sporangia, and necropsies ( $n = 2$ ) confirmed that lesions were confined to the skin.<sup>7</sup> Dermocystid 18S rRNA sequences ( $n = 4$ ) from both salamander species were identical. Phylogenetic analysis revealed a close relationship to *Dermotheca* [*Amphibiocystidium*] *viridescens*, a dermocystid affecting newts from the eastern United States.<sup>6</sup> Dermocystids were not identified in silvery salamander museum specimens from the same wetlands ( $n = 125$ ) dating back to 1973. This is the first report of *Dermotheca* sp. affecting caudates in the Midwestern United States. Further research is needed to determine the health effects and conservation implications of this parasite.

**Key words:** *Ambystoma platineum*, *Ambystoma texanum*, *Dermotheca* sp., Mesomycetozoa, silvery salamander, small-mouthed salamander

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## CHARACTERIZING THE CLINICAL COURSE OF AN EMERGING FUNGAL PATHOGEN (*Emydomyces testavorans*) IN THE STATE-ENDANGERED ALLIGATOR SNAPPING TURTLE (*Macrochelys temminckii*)

Laura Adamovicz, DVM, PhD,<sup>1\*</sup> Daniel B. Woodburn, DVM, DACVP,<sup>2</sup> Kayla Boers, DVM,<sup>1</sup> Samantha Johnson, BS,<sup>1</sup> Taylor Willis, BS,<sup>1</sup> Ethan Kessler, BS,<sup>3</sup> and Matthew C. Allender, DVM, MS, PhD, Dipl ACZM<sup>1</sup>

<sup>1</sup>Wildlife Epidemiology Laboratory, University of Illinois College of Veterinary Medicine, Urbana, IL 61802 USA; <sup>2</sup>Zoological Pathology Program, University of Illinois College of Veterinary Medicine, Urbana, IL 61802 USA; <sup>3</sup>Illinois Natural History Survey, Prairie Research Institute, Champaign, IL 61820 USA

### Abstract

Fungal infections contribute to significant morbidity and mortality in reptiles. *Emydomyces testavorans* is an emerging fungal pathogen associated with ulcerative shell disease in multiple chelonians, including species of conservation concern.<sup>1</sup> While *E. testavorans* morphology, growth, and molecular phylogeny have been described, characteristics of lesion progression are unknown.<sup>1</sup> We report the clinical course of *E. testavorans* infection in a cohort of juvenile alligator snapping turtles (AST; *Macrochelys temminckii*). Fourteen ASTs presented for evaluation in October 2016 following the death of one animal. Initial clinical signs included paronychia and nail loss ( $n = 9$ ), cutaneous ulceration ( $n = 7$ ), plastron ulceration ( $n = 6$ ), and rhinitis ( $n = 5$ ). *E. testavorans* was diagnosed using polymerase chain reaction (PCR) and culture, and all turtles tested negative for ranavirus, herpesvirus, and *Mycoplasma* sp. Seven turtles died or were euthanized due to progressive clinical signs. Necropsy and histopathology revealed ulcerative dermatitis/rhinitis ( $n = 8$ ) and rare osteomyelitis ( $n = 1$ ), with no other significant findings. Lesions were PCR positive for *E. testavorans* and were colonized with morphologically consistent fungi. Most clinical signs in survivors resolved within several months; however, over the next 3 yr turtles developed progressive hyperkeratosis with excessive skin and scute shedding, and experienced intermittent cutaneous ulceration of the extremities. All turtles remain *E. testavorans* PCR positive. This report illustrates that *E. testavorans* can cause significant cutaneous lesions in ASTs and demonstrates that spontaneous clearance is unlikely. *E. testavorans* should be considered a pathogen of concern for AST head-starting and reintroduction programs.

**Key words:** Alligator snapping turtle, *Emydomyces testavorans*, fungal dermatitis, *Macrochelys temminckii*, reptile

### ACKNOWLEDGMENTS

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## DIAGNOSIS AND TREATMENT OF CHAGAS DISEASE (*Trypanosoma cruzi*) IN A NATURALLY INFECTED DE BRAZZA'S MONKEY (*Cercopithecus neglectus*) IN ALABAMA

*Stephanie McCain, DVM, Dipl ACZM,<sup>1\*</sup> Richard R. Sim, DVM, Dipl ACZM,<sup>2</sup> Bridget Weidner, LVT,<sup>1</sup> and Sarah Hamer, MS, PhD, DVM, Dipl ACVPM<sup>3</sup>*

<sup>1</sup>Birmingham Zoo, Inc., Birmingham, AL 35223 USA; <sup>2</sup>Oregon Zoo, Portland, OR 97221 USA; <sup>3</sup>College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843 USA

### Abstract

*Trypanosoma cruzi*, the causative agent of Chagas disease, is a zoonotic, vector-borne parasite with a wide host range. While natural infections have been documented in both wild and captive nonhuman primates, there are no reports of treatment.<sup>2-6</sup> An 11-yr-old captive-bred male De Brazza's monkey (*Cercopithecus neglectus*) presented with weight loss, despite normal appetite in August, 2018. Physical exam findings under anesthesia included pale mucous membranes and thin body condition. Two-view chest and abdominal radiographs and abdominal ultrasound were unremarkable. A complete blood count (CBC) revealed a normal leukogram and non-regenerative anemia (17%), and a biochemistry panel revealed severe hypoglycemia (26 mg/dl) in the face of low insulin (< 7.5 µU/ml.) The monkey was treated with supportive care and had an uncomplicated anesthetic recovery. Many trypanosomes were seen on a blood smear and a blood sample was polymerase chain reaction (PCR) positive for *T. cruzi*. Strain typing revealed parasite discrete typing unit TcIV. The monkey was treated with Benznidazole (Exeltis USA, Inc., Florham Park, New Jersey 07932 USA) 25 mg p.o., b.i.d. for 60 days with no obvious negative effects. Blood was initially obtained daily, then weekly, then monthly through voluntary blood collection to monitor CBC, biochemistry panel, cardiac troponin, quantitative PCR, and serology; obtaining voluntary blood samples was instrumental in monitoring his response to treatment. Over several months, serology demonstrated a rise in immunoglobulin G and a decrease in immunoglobulin M and qPCR showed a decrease. Mild hypoglycemia persisted for several months, which has been documented in other species infected with trypanosomes.<sup>1,7</sup> The monkey recovered with no apparent lasting effects.

**Key words:** Benznidazole, *Cercopithecus neglectus*, Chagas disease, DeBrazza's monkey, *Trypanosoma cruzi*

### ACKNOWLEDGMENTS

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## LEUKOCYTE CHARACTERISTICS OF THE GIANT PANDA (*Ailuropoda melanoleuca*): MORPHOLOGIC, CYTOCHEMICAL AND ULTRASTRUCTURAL FEATURES

*Spencer Kehoe, DVM,<sup>1\*</sup> Nicole I. Stacy, DVM, DrMedVet, DACVP,<sup>2</sup> Salvatore Frasca Jr, VMD, PhD, DACVP,<sup>2</sup> Tracy Stokol, BVSc, PhD, DACVP,<sup>3</sup> Chengdong Wang, MS,<sup>5</sup> Sharon T. Debose, BS, MEd, RVT,<sup>1</sup> Kate Leach, BVSc,<sup>1</sup> Li Luo, MS,<sup>5</sup> and Sam Rivera, DVM, MS, DABVP (Avian Practice), Dipl ACZM, DipECZM (Zoo Health Management)<sup>1</sup>*

*<sup>1</sup>Department of Veterinary Services, Zoo Atlanta, Atlanta, GA 30315 USA; <sup>2</sup>Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 USA; <sup>3</sup>Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY 14853 USA; <sup>4</sup>Chengdu Research Base of Giant Panda Breeding, Northern Suburb Chengdu, Sichuan 610081 P.R. China*

### Abstract

The giant panda (*Ailuropoda melanoleuca*) is a vulnerable species and a charismatic, prominent member of zoological collections worldwide. Despite its importance, few studies have been conducted to determine the hematologic reference of this species,<sup>1,2</sup> and no studies to date have been performed to describe normal cell morphology or constituents, achieved by traditional techniques such as cytochemical staining and evaluation of ultrastructural features.<sup>3-5</sup> The objective of this study was to characterize giant panda leukocytes using a routine modified Wright's stain, eight cytochemical stains, and transmission electron microscopy (TEM) to further the understanding of accurate cell identification and cytoplasmic contents. Voluntary venipuncture was performed on four healthy individual animals (two adults and two juveniles). Blood was collected from the cephalic vein into calcium ethylenediaminetetraacetic acid (Ca<sup>2+</sup> EDTA) for routine and cytochemical stains, and into 2.5% glutaraldehyde preservative for TEM. On routinely stained blood smears, leukocytes could be differentiated into granulocytes (neutrophils, eosinophils, and basophils) and mononuclear cells (lymphocytes and monocytes). Cytochemical staining revealed similar leukocyte staining patterns to that seen in other mammals, with some species differences. On TEM, leukocytes with nuclear and cytoplasmic features of mononuclear cells (e.g., lymphocytes and monocytes) were differentiated from granulocytes, which had a lobulated nucleus and cytoplasmic granules of different electron densities. This study provides novel information on giant panda morphology and cellular constituents and will be clinically applicable for the establishment of hematologic reference intervals, as well as provide a baseline for understanding leukocyte changes in response to disease.

**Key words:** *Ailuropoda melanoleuca*, cytochemistry, giant panda, hematology, leukocyte morphology, transmission electron microscopy

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## SWIMMER SYNDROME IN SNOW LEOPARD (*Panthera uncia*) CUBS: A SERIES OF FOUR CASES

*John M. Sykes IV, DVM, Dipl ACZM,<sup>1\*</sup> Jenessa Gjeltema, DVM, Dipl ACZM,<sup>2,3</sup> Anne Rivas, DVM,<sup>1,4</sup> Ray Wack, DVM, Dipl ACZM,<sup>2,3</sup> Victoria L. Clyde, DVM,<sup>5</sup> Christy L. Rettenmund, DVM, Dipl ACZM,<sup>5</sup> Sean Brady, DVM,<sup>6</sup> Denis J. Marcellin-Little, DEDV,<sup>7</sup> Erika Gebhard, DVM,<sup>6</sup> and Leilani Alvarez, DVM, Dipl ACVSMR<sup>8</sup>*

<sup>1</sup>*Wildlife Conservation Society, Zoological Health Program, Bronx, NY 10460 USA;*

<sup>2</sup>*Department of Veterinary Medicine and Epidemiology and the Karen C. Drayer Wildlife Health Center, University of California Davis School of Veterinary Medicine, Davis, CA*

*95616 USA;* <sup>3</sup>*Sacramento Zoo, Sacramento, CA 95822 USA;* <sup>4</sup>*Current address: Birmingham Zoo, Birmingham, AL 35223 USA;* <sup>5</sup>*Milwaukee County Zoo, Milwaukee, WI 53226 USA;*

<sup>6</sup>*San Francisco Zoo and Gardens, San Francisco, CA 94132 USA;* <sup>7</sup>*University of California Davis School of Veterinary Medicine, Davis, CA 95616 USA;* <sup>8</sup>*Animal Medical Center, New York, NY 10065 USA*

### Abstract

Swimmer syndrome<sup>1</sup> was diagnosed in four (two male, two female) snow leopard (*Panthera uncia*) cubs. Litter size ranged from one to two, but two of the twin cubs died at 2-3 days of age, resulting in a rearing litter size of one for three cases. Age at diagnosis was 14 to 35 days. Clinical signs included splayed rear legs (4/4 cases), superficial abrasions (4/4), dorsoventrally flattened thorax (3/4), tarsal rotation (3/4), and splayed front legs (1/4). Two cubs also had bilateral eyelid colobomas. One cub developed a respiratory infection suspected to be a consequence of the abnormal chest conformation. All four cubs were mother-reared and received rehabilitation therapy 1-4 times per day. Three animals were removed from the dam, treated, and returned after each session; one animal was removed from the dam in the AM and returned the late PM. Therapy included use of corrective devices (hobbles, shoes, or splints; 4/4), encouraged activity on varied substrate (4/4), joint compressions/standing with support (3/4), walking with support/sling (3/4), tunnel/chute walking (3/4), range of motion and massage (2/4), and toe pinch/withdrawal exercises (1/4). Length of treatment was 52 to 88 days (ages 73-123 days). All cubs were ultimately able to walk unassisted, though three had mild abnormalities persist (rear leg lameness, rotated pelvic limbs, abducted pelvic limbs, and lax coxofemoral joints). Predisposing factors may have included slick flooring and a heavy body condition. This severe condition may be corrected with intensive care and cubs may be left with the dam for rearing.

**Key words:** *Panthera uncia*, rehabilitation, swimmer syndrome

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## CARDIAC ASSESSMENT AND USE OF IMPLANTABLE LOOP RECORDERS IN THREE CAPTIVE MALE GELADAS (*Theropithecus gelada*)

*Stephanie H. Zec, DVM,<sup>1\*</sup> Marc Miller, MD,<sup>2</sup> Lori Croft, MD,<sup>2</sup> Martin Goldman, MD,<sup>2</sup> Jessica Moody, PhD,<sup>1</sup> Colleen McCann, PhD,<sup>1</sup> and Susan L. Bartlett, DVM, Dipl ACZM<sup>1</sup>*

*<sup>1</sup>Bronx Zoo, Wildlife Conservation Society, Bronx, NY 10460 USA; <sup>2</sup>Mount Sinai Heart, Mount Sinai Medical Center, New York, NY 10029 USA*

### Abstract

Cardiac disease is a major cause of morbidity and mortality in primates.<sup>1-3,7,9</sup> Besides cardiomyopathy associated with vitamin E deficiency, there is limited information regarding cardiac disease in geladas (*Theropithecus gelada*).<sup>5</sup>

Arrhythmias can be difficult to diagnose in primates as clinical signs are rarely observed prior to death. Anesthetized electrocardiograms (ECGs) have limited diagnostic value if arrhythmias are intermittent, and can be influenced by anesthetic agents. Implantable loop recorders (ILRs), are surgically placed devices that allow for ECG monitoring in awake animals. ILRs have demonstrated promise in reliably detecting arrhythmias in small domestic animals and chimpanzees (*Pan troglodytes*).<sup>4,6,8</sup>

At the Bronx Zoo between 2007 and 2018 there were ten incidents of sudden cardiac death in male geladas, none of which showed premonitory signs. Examinations of three adult male geladas exhibiting no clinical signs included thoracic radiographs, echocardiograms, 12-lead ECGs, and subcutaneous surgical placement of Reveal LINQ Insertable Cardiac Monitors (Medtronic, Minneapolis, MN 55432-5604 USA). Serum lipid profiles, vitamin E, troponin, and complete blood work was performed. Wireless ILR downloads were obtained during behavioral training or automatically when the animal slept within 15 feet of the bluetooth receiver. One male had an elevated troponin level (210 ng/L) and a reduced ejection fraction (46%). Additionally in that same individual, ILR downloads indicated supraventricular tachycardia and non-sustained ventricular tachycardia. Atrial tachycardia, as well as sinus tachycardia and bradycardia, were observed in the other two individuals. The use of the ILR has the potential to revolutionize our ability to diagnose and treat arrhythmias in primates and other zoological species.

**Key words:** Arrhythmia, cardiovascular disease, gelada, implantable loop recorders, *Theropithecus gelada*

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## CARDIAC EVALUATION IN WESTERN LOWLAND GORILLAS (*Gorilla gorilla gorilla*): COMPARATIVE ASSESSMENT OF B-TYPE NATRIURETIC PEPTIDES FROM URINE, CAPILLARY SERUM, AND VENOUS SERUM SAMPLES

*Taylor J. Yaw, DVM, CertAqV,<sup>1,2\*</sup> Frances M. Moore, DVM, DACVP,<sup>3</sup> Kurt K. Sladky, MS, DVM, Dipl ACZM, DipECZM (Herpetology),<sup>1</sup> Marietta D. Danforth, BS, PhD,<sup>4</sup> Hayley W. Murphy, DVM,<sup>4</sup> and Victoria L. Clyde, DVM<sup>2</sup>*

<sup>1</sup>*Department of Surgical Sciences, University of Wisconsin School of Veterinary Medicine, Madison, WI 53706 USA;* <sup>2</sup>*Department of Animal Health, Milwaukee County Zoo, Milwaukee, WI 53226 USA;* <sup>3</sup>*Veterinary Services, Marshfield Labs, Marshfield, WI 54449 USA;* <sup>4</sup>*Great Ape Heart Project, Zoo Atlanta, Atlanta, GA 30315 USA*

### Abstract

Cardiac disease is a significant cause of morbidity and mortality in great apes, including Western lowland gorillas (*Gorilla gorilla gorilla*).<sup>2</sup> Antemortem detection of cardiac disease can be difficult due to the lack of definitive clinical signs prior to acute death, and detection currently relies on advanced diagnostics requiring anesthesia in gorillas with unknown cardiac status.<sup>1</sup> The development and application of biochemical markers, which are capable of detecting and staging cardiac disease, without the animal undergoing general anesthesia, are warranted. In this study, amino-terminal pro B-type natriuretic peptide (NT-proBNP) was measured in capillary serum, urine, and venous serum samples from Western lowland gorillas ( $n = 26$ ) during routine immobilizations ( $n = 28$ ) involving seven zoological facilities. Capillary serum samples were collected via a vacuum collection system (Comedo Suction Microdermabrasion Machine, KRASR, Mississauga, Ontario L4Z 1V9 Canada and Innovac Quick-Draw, Innovative Med Tech, Leawood, Kansas 66224 USA) after application of a lancet (Feather Blood Lancet, GF Health Products Incorporated, Atlanta, Georgia 30340 USA) to the lateral aspect of the first pelvic limb digit, while urine samples were collected via cystotomy, urethral catheterization, or free catch within 24 hr of the immobilization event. In addition, validation of NT-proBNP recovery from gorilla urine was performed as part of this study. Capillary serum and urine NT-proBNP levels were tested for correlation with venous serum samples and with echocardiographic diagnosis when available. The results of this study provide a novel strategy for capillary blood collection in Western lowland gorillas, and will improve standards for the diagnosis and management of gorillas with cardiac disease in captive collections.

**Key words:** Amino-terminal pro B-type natriuretic peptide, capillary serum, cardiac disease, *Gorilla gorilla gorilla*, Western lowland gorilla

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## ELECTROCHEMOTHERAPY FOR THE TREATMENT OF ORAL SQUAMOUS CELL CARCINOMA IN AN ASIAN SMALL-CLAWED OTTER (*Aonyx cinereus*)

**Kailey B. Anderson, DVM,<sup>1\*</sup> Jessica Siegal-Willott, DVM, Dipl ACZM,<sup>1</sup> Christine H. Merrick, DVM, DACVIM (Oncology),<sup>2</sup> and Amanda Foskett, DVM, DACVIM (Oncology)<sup>3</sup>**

<sup>1</sup>Smithsonian National Zoological Park, Washington, DC 20008 USA; <sup>2</sup>Katonah Bedford Veterinary Center, Bedford Hills, NY 10507 USA; <sup>3</sup>Friendship Hospital for Animals, Washington, DC 20016 USA

### Abstract

A 7-yr-old female Asian small-clawed otter (*Aonyx cinereus*) was evaluated for an oral mass and 2-wk history of altered chewing behavior. Biopsy and histopathology revealed a diagnosis of oral squamous cell carcinoma (SCC). Diagnostic computed tomography (CT) revealed a non-resectable, right-sided mass involving the tongue, ventral pharynx, and hyoid bones. Treatment options for oral SCC include surgical resection, radiation therapy, chemotherapy, or palliative care with non-steroidal anti-inflammatories.<sup>1,3,5</sup> More recently, electrochemotherapy (ECT) was used for treatment of oral SCC in domestic dogs resulting in marked tumor responses (CM personal observation), and was used for treatment of cutaneous SCC and cutaneous papillomas in turtles.<sup>2,4</sup> In this case, initial treatment included meloxicam (Meloxicam, Ceva Animal Health LLC, Lenex, Kansas 66215 USA) 0.1 mg/kg p.o. s.i.d followed by three ECT treatments every 2-4 wk. For each ECT treatment, bleomycin (Bleomycin, Meitheal Pharmaceuticals, Chicago, Illinois 60631 USA) 20 mg/m<sup>2</sup> was administered intravenously 10 min prior to intralesional cisplatin (Cisplatin, Alvogen Inc, Pine Brook, New Jersey 07058 USA) 0.2 mg/kg and delivery of biphasic electric pulses using reversible and irreversible electroporation. After treatment, the rostral aspect of the tumor regressed visibly, but recheck CT confirmed a poor response at the caudal aspect. ECT was discontinued due to the small size of the oral cavity precluding effective access and treatment of the caudal aspect of the tumor. Two months after the last ECT treatment and 7 mo after initial evaluation, the patient is doing well. ECT was effective in providing palliation, as well as marked tumor regression in the rostral aspect of the lesion. ECT should be considered in the treatment of oral SCC where the tumor is accessible to administration of ECT.

**Key words:** *Aonyx cinereus*, Asian small-clawed otter, carcinoma, electrochemotherapy, oncology

### ACKNOWLEDGMENTS

The authors would like to thank the curators and keepers at the National Zoological Park who cared for this animal.

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## DIAGNOSIS AND MEDICAL MANAGEMENT OF PULMONARY ACARIASIS IN TWO SILVERBACK WESTERN LOWLAND GORILLAS (*Gorilla gorilla*) AT DIFFERENT ZOOLOGICAL INSTITUTIONS

*James G. Johnson III, DVM, MS, CertAqV, Dipl ACZM,<sup>1\*</sup> Sathya K. Chinnadurai, DVM, MS, Dipl ACZM, Dipl ACVAA, Dipl ACAW,<sup>2</sup> Gregory E. Holt, MD, PhD,<sup>3</sup> Marisa Bezjian, DVM,<sup>1</sup> Gabriella L. Flacke, DVM, MVSc, PhD,<sup>1</sup> Heather Stockdale Walden, MS, PhD,<sup>4</sup> Jennifer N. Langan, DVM, Dipl ACZM, DipECZM (Zoo Health Management),<sup>2,5</sup> Julie Sheldon, DVM, MS,<sup>2</sup> and Gwen E. Myers, DVM<sup>1</sup>*

<sup>1</sup>*Department of Animal Health, Zoo Miami, Miami, FL 33177 USA;* <sup>2</sup>*Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513 USA;* <sup>3</sup>*Department of Internal Medicine, Miller School of Medicine, University of Miami, Miami, FL 33136 USA;* <sup>4</sup>*Department of Comparative, Diagnostic and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL 32608 USA;* <sup>5</sup>*Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 USA*

### Abstract

Pulmonary acariasis caused by mites in the genus *Pneumonyssus* has historically been described in Old World monkeys and chimpanzees (*Pan troglodytes*);<sup>1</sup> however, reports of pulmonary acariasis have been scant in recent literature and this condition has not been reported in gorillas in North American zoos. A 25-yr-old male western lowland gorilla (*Gorilla gorilla*) at Zoo Miami was diagnosed with pulmonary acariasis caused by mites consistent with *Pneumonyssus* sp. identified using bronchoscopy following a 2-yr history of intermittent coughing since arrival that was not responsive to any specific empirical treatments. The animal was treated with oral ivermectin (Noromectin 1% solution, Norbrook® Inc., Overland Park, Kansas 66219 USA) 0.2 mg/kg p.o. every 2 wk administered with prednisolone (PrednisTab 20 mg, LLOYD, Inc., Shenandoah, Iowa 51601 USA) 0.2 mg/kg p.o. and clinically improved 2 mo following treatment, but still had intermittent mild residual coughing. A 39-yr-old male western lowland gorilla at the Brookfield Zoo was diagnosed with pulmonary acariasis following a 7-yr history of intermittent coughing that was managed with inhaled steroids and antihistamines. Previous bronchoscopy only showed inflammation, but bronchoscopy 4 yr later revealed mites in the lower respiratory tract. The animal was treated with oral ivermectin (0.2 mg/kg p.o.) monthly for 2 mo and no mites were seen at recheck bronchoscopy. The life cycle of pulmonary mites in primates is unknown but infection in these gorillas was suspected to be due to spread from other primates (Old World monkeys, chimpanzees) in proximity that may have harbored subclinical infection. This is the first report of pulmonary acariasis in gorillas residing in North America, and this condition should be considered and screened for in apes with chronic coughing.

**Key words:** Bronchoscopy, cough, *Gorilla gorilla*, mite, *Pneumonyssus*, pulmonary acariasis

### ACKNOWLEDGMENTS

We thank the animal care and veterinary technician staff at Zoo Miami and Brookfield Zoo for their care of these animals.

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## TREATMENT OF THYROID DISORDER IN AN ADULT FEMALE ATLANTIC WALRUS (*Odobenus rosmarus rosmarus*)

Claire Vergneau-Grosset,<sup>1,2\*</sup> Stéphane Lair,<sup>1,2</sup> Mario Guay,<sup>1</sup> Hugo Joly, and Noémie Summa<sup>1,2</sup>

<sup>1</sup>Faculté de médecine vétérinaire, Université de Montréal, St. Hyacinthe, Québec, J2S 8H5, Canada; <sup>2</sup>Aquarium du Québec, Québec City, Québec, G1W 4S3, Canada

### Abstract

A 15-yr-old aquarium-housed female Atlantic walrus (*Odobenus rosmarus rosmarus*) presented with a non-painful cervical enlargement that had progressed over a few months. Cervical ultrasound showed bilateral masses, located 2.5 to 5 cm from the skin surface and non-adherent to deep planes. These masses were composed of a cystic and vascularized glandular tissue, delineated by a well-demarcated capsule. Within 10 mo, the lesion's size increased from 10 cm to greater than 25 cm in diameter. Repeated fine needle aspirations were inconclusive. Hematology and biochemistry were unremarkable. Total T4 concentrations (9.2 and 9.6 nmol/L) were markedly lower than values obtained in wild walruses (unpublished data). Serum TSH levels (IMMULITE®, Diagnostic Products Corporation, Los Angeles, CA, 90045-6900, USA) of 7.51 ng/ml were markedly increased compared to values obtained in three other aquarium-housed walruses (0.06 - 1.01 ng/ml,  $n = 7$  samples). Hypothyroidism associated with hyperplastic thyroid glands was suspected. Nutritional factors including iodine and selenium intake were evaluated. Dietary modifications were implemented including a switch to a mineral supplement without iodine. Treatment with levothyroxine was initiated with follow-up TSH level measurements for dose adjustment. A maintenance dose of 0.02 mg/kg orally twice daily produced a reduction of TSH concentration to 0.27 ng/ml. Mass diameter decreased to 10 cm in diameter within 4 mo; however, hormonal factors or immunity modulation associated with pregnancy cannot be ruled out as this female was pregnant during treatment. Even if not reported in the scientific literature, this case, as well as anecdotal reports, suggests that thyroid hyperplastic lesions should be considered in the presence of cervical enlargement in this species.

**Key words:** Iodine, levothyroxine, *Odobenus rosmarus*, thyroid, TSH, walrus

### ACKNOWLEDGMENTS

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## HEALTH ASSESSMENT AND TRANSLOCATION OF FREE-RANGING BOLIVIAN RIVER DOLPHINS (*Inia boliviensis*) EXPERIENCING HIGH ANTHROPOGENIC DISTURBANCES

*Julie D. Sheldon, DVM,<sup>1,2\*</sup> Sharon L. Deem, DVM, PhD, Dipl ACZM,<sup>3</sup> Claudia Venegas Cuzmar, Blg,<sup>4,5</sup> Kathia Rivero,<sup>4</sup> Paulo Colchao, BMVZ,<sup>6</sup> Paul A. Van Damme,<sup>7</sup> and Ellen Bronson, med vet, Dipl ACZM<sup>8</sup>*

*<sup>1</sup>Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513 USA; <sup>2</sup>Illinois Zoological and Aquatic Animal Residency Program, Urbana, IL 61802 USA; <sup>3</sup>Saint Louis Zoo Institute for Conservation Medicine, Saint Louis, MO 63110 USA; <sup>4</sup>Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia; <sup>5</sup>Gobierno Autonomo Departamental Santa Cruz, Santa Cruz de la Sierra, Bolivia; <sup>6</sup>Royal Veterinary College, University of London, London, United Kingdom, NW1 0TU; <sup>7</sup>Faunagua, Cochabamba, Bolivia; <sup>8</sup>The Maryland Zoo in Baltimore, Baltimore, MD 21217 USA*

### Abstract

The endangered Bolivian river dolphin (*Inia boliviensis*) is increasingly threatened by agricultural river modifications, which can cause stranding in shallow water or isolation into lagoons separated from the river.<sup>1-3</sup> Intensive soybean field management using unknown levels of pesticides and contaminants is transforming the natural riparian forest. Approximately 18 dolphins have been isolated from the Rio Grande in a lake since 2012, which has decreased in size from 26 km<sup>2</sup> in 1986 to 3 km<sup>2</sup> in 2016.<sup>2</sup> Using nets, manual capture, and truck transportation, we translocated six dolphins (five male, one female) from the lake back to the Rio Grande. Each dolphin underwent physical examination, diagnostic sampling, and satellite tag placement ( $n = 3$ ). Diagnostic testing included capture and release blood gas analysis; complete blood count; serum biochemistry; blowhole, urogenital, oral and fecal cytology  $\pm$  culture; and fecal parasite examination. Capture to release took on average 4.8 hr and all dolphins tolerated the process without complications. Significant findings included minor skin lesions, healed fractures, dental morphology variations, and fecal trichurids and strongyles. Clinical pathology results were unremarkable. *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella oxytoca* were cultured from oral, fecal and/or blowhole samples. Future work includes monitoring movements via satellite, *Brucella* and *Leptospira* serology, serum pollutant concentration, translocating remaining dolphins, and public education. Living at the intersection of natural rivers, agricultural expansion, and human settlement, Bolivian river dolphins are sentinels of ecosystem health. This and this project's team diversity, from villagers to veterinarians, emphasize the importance of a One Health approach to conservation.

**Key words:** Bolivian river dolphin, cetacean, health assessment, *Inia boliviensis*, One Health, translocation

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## CLINICAL APPLICATIONS OF PINNIPED BONE MARROW EVALUATION: COLLECTION TECHNIQUES AND PRELIMINARY ANALYSES

*Cara L. Field, DVM, PhD, Dipl ACZM,<sup>1\*</sup> Fernanda M. Carpintero, MV,<sup>2</sup> Vitor Luz Carvalho, MVB, Dr,<sup>3</sup> Carlos A. Rios,<sup>1</sup> Pádraig J. Duignan, MVB, PhD,<sup>1</sup> and Nicole I. Stacy, DVM, DrMedVet, DACVP<sup>4</sup>*

*<sup>1</sup>The Marine Mammal Center, Sausalito, CA 94965 USA; <sup>2</sup>Aiuká Consultoria em Soluções Ambientais, Rio das Ostras/RJ Brazil; <sup>3</sup>Associação de Pesquisa e Preservação de Ecossistemas Aquáticos, Caucaia/CE, Brazil; <sup>4</sup>Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 USA*

### Abstract

As diving mammals subjected to routine dramatic pressure changes, marine mammals produce large erythrocytes with comparatively higher oxygen carrying capacity and blood volume than terrestrial species, however marine mammal hematopoiesis has been minimally investigated to date. Bone marrow evaluation is critical in the assessment of hematopoiesis and the optimal collection site varies among species based on anatomic and physiologic differences and safety. We sought to determine optimal bone marrow sampling site(s) and to cytologically and histopathologically characterize this tissue in pinnipeds. Bone marrow aspirates and biopsies were collected from the dorsal iliac crest and tibial crest from two clinically ill otariids while under anesthesia; and at time of euthanasia (12 otariids; one phocid) using an 11-gauge Jamshidi™ needle and/or 14, 16, or 18 gauge needles. Pinnipeds euthanized due to severe disease included animals with neoplasia, leptospirosis, domoic acid intoxication, or congenital abnormality; sampling sites from euthanized animals were evaluated at necropsy to assess techniques. Samples were collected into EDTA or heparin and slides were prepared immediately from full aspiration samples and/or aspiration of bone spicules from a Petri dish. Aspirates showed systemic inflammatory responses in the myeloid cell line ( $n = 11$ ), bacterial infection ( $n = 3$ ), lymphoid and/or plasma cell infiltrates ( $n = 7$ ), erythroid cell line changes ( $n = 2$ ), assessment of megakaryocytes ( $n = 15$ ), and occasional presence of microfilaria ( $n = 9$ ). Superior quality samples were obtained with a ratio of 3 ml EDTA to 1 ml bone marrow. These results provide valuable information on bone marrow collection techniques and cytologic and histopathologic evaluation methods in pinniped species.

**Key words:** Bone marrow, hematopoiesis, pinniped

### ACKNOWLEDGMENTS

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## DEVELOPMENT OF A MODIFIED GLASGOW COMA SCALE FOR RAPTORIAL SPECIES WITH HEAD TRAUMA

*Sarah K. Reich, DVM,<sup>1\*</sup> Krista A. Keller, DVM, Dipl ACZM,<sup>1,3</sup> Devon Hague, DVM, DACVIM (Neurology),<sup>2</sup> Kari Foss, DVM, DACVIM (Neurology),<sup>2</sup> Matthew C. Allender, DVM, MS, PhD, Dipl ACZM,<sup>1,3</sup> and Julia Whittington, DVM<sup>1</sup>*

*<sup>1</sup>Zoological Medicine, Department of Veterinary Clinical Medicine, University of Illinois, College of Veterinary Medicine, Urbana, IL 61802 USA; <sup>2</sup>Neurology and Neurosurgery, Department of Veterinary Clinical Medicine, University of Illinois, College of Veterinary Medicine, Urbana, IL 61802 USA; <sup>3</sup>Wildlife Epidemiology Laboratory, Department of Veterinary Clinical Medicine, University of Illinois, College of Veterinary Medicine, Urbana, IL 61802 USA*

### Abstract

The Glasgow Coma Scale, widely used in human medicine, is a simple and practical method for assessment of impaired neurologic status.<sup>1,2</sup> The objectives of this study were to: 1) develop a Modified Glasgow Coma Scale (MGCS) for use in raptors presenting with head trauma and 2) assess the agreement of MGCS scores between examiners with varying experience backgrounds. Seventeen native raptors presenting to the University of Illinois Wildlife Medical Clinic were included in this prospective study based upon clinical signs or history suggestive of head trauma. All raptors received a MGCS assessment by three individuals within 8 hr of presentation: a veterinary student, a wildlife veterinarian, and a board-certified or resident veterinary neurologist. Each animal received a score (1-5) in three separate categories: motor activity, level of consciousness, and brainstem reflexes. An overall score was tabulated from the categories. Agreement between the three individual scores was assessed via Cronbach's alpha and intraclass correlation. There was excellent-good agreement in all three assessment categories as well as overall score, indicating that the MGCS would be comparable between different experience backgrounds. As the initial assessment of patients in wildlife rehabilitation may not always be performed by a veterinarian, it is important that any evaluation tools be practical for multiple experience levels. The ability to rapidly and reliably determine prognosis in patients can also help in the assessment of quality of life while undergoing rehabilitation. Further research is underway evaluating the potential prognostic value of the MGCS in raptor species with head trauma.

**Key words:** Bird, hawk, owl, prognosis, rehabilitation, wildlife

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## FACTORS AFFECTING ABNORMAL MOLTING IN THE MANAGED AFRICAN PENGUIN (*Spheniscus demersus*) POPULATION IN NORTH AMERICA

*Matthew Golembeski, DVM,<sup>1\*</sup> Samantha J. Sander, DVM, Dipl ACZM,<sup>2,3</sup> Jennifer Kottyan, BS,<sup>2</sup> and William E. Sander, DVM, MPH, Dipl ACVPM<sup>3</sup>*

<sup>1</sup>*Michigan State University College of Veterinary Medicine, East Lansing, MI 48824 USA;*  
<sup>2</sup>*The Maryland Zoo in Baltimore, Baltimore, MD 21217 USA;* <sup>3</sup>*University of Illinois College of Veterinary Medicine, Urbana, IL 61802 USA*

### Abstract

Abnormal molting, including partial or incomplete molt, arrested molt cycle, or inappropriate frequency of molt, is a primary concern for the managed African penguin (*Spheniscus demersus*) population and is documented across institutions.<sup>1,2</sup> In order to identify risk factors for abnormal molts and characterize intervention opportunities, a comprehensive survey evaluating numerous husbandry and medical parameters was created. Results of this survey represent 45 African penguin holding facilities and 736 unique animals. Of those individuals, 135 (18.3%) demonstrated an abnormal molt over the 5-yr study period. Identified risk factors included advanced age and facilities utilizing freshwater pools. Normally molting penguins were more commonly housed with saltwater pool access and natural lighting cycles. Current treatment for inducing molt in African penguins, including hormonal regulation, dietary modification, and holistic interventions, is anecdotal and has been met with variable success. Subcutaneous 5.4 mg melatonin implants placed in anticipation of environmental molting cues showed the most promise at inducing catastrophic molt, with 14/17 (82.3%) of affected individuals molting normally following this treatment. Contrary to reports in other penguin species, thyroxine and progestin treatments were rarely associated with molting in the birds in this study.<sup>3-5</sup> Survey analysis indicated that abnormal molt is a complex, multifactorial process, but modifiable factors exist that may predispose animals to abnormally molt. Addressing these factors in future exhibit designs may mitigate the prevalence of this condition. Despite these efforts, it is likely that medical interventions will be required to aid in the treatment of abnormal molting in this species.

**Key words:** African penguin, husbandry, melatonin, molting, *Spheniscus demersus*

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**CIRCULATING NUTRIENTS IN FREE-LIVING HUMBOLDT PENGUINS (*Spheniscus humboldti*), GUANAY CORMORANTS (*Phalacrocorax bougainvilliorum*), AND PERUVIAN PELICANS (*Pelecanus thagus*) FROM PUNTA SAN JUAN, PERU**

*Hillary E. Allgood, MS,<sup>1,2\*</sup> Michael J. Adkesson, DVM, Dipl ACZM, DipECZM (Zoo Health Management),<sup>3</sup> Matthew C. Allender, DVM, MS, PhD, Dipl ACZM,<sup>4</sup> Andrea J. Fascetti, VMD, PhD,<sup>5</sup> Susana Cárdenas-Alayza, MSc,<sup>6</sup> Marco Cardeña,<sup>6</sup> Ellen S. Dierenfeld, PhD,<sup>7</sup> and Sharon L. Deem, DVM, PhD, Dipl ACZM<sup>1</sup>*

<sup>1</sup>*Institute for Conservation Medicine, Saint Louis Zoo, Saint Louis, MO 63110 USA;* <sup>2</sup>*College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 USA;* <sup>3</sup>*Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513 USA;* <sup>4</sup>*Wildlife Epidemiology Laboratory, University of Illinois, College of Veterinary Medicine, Urbana IL, 61802 USA;* <sup>5</sup>*Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616 USA;* <sup>6</sup>*Punta San Juan Program, Center for Environmental Sustainability, Cayetano Heredia University, Lima, Peru;* <sup>7</sup>*Ellen S. Dierenfeld, LLC Comparative Animal Nutrition Consulting, Saint Louis, MO 63128 USA*

**Abstract**

Resource competition with commercial fisheries and changes in prey availability related to El Niño Southern Oscillation (ENSO) events have adversely effected Humboldt penguins (HP, *Spheniscus humboldti*), Guanay cormorants (GC, *Phalacrocorax bougainvilliorum*), and Peruvian pelican (PP, *Pelecanus thagus*) populations within the Humboldt Current marine ecosystem along coastal Peru. Ecosystem effects from environmental and human impacts can result in decreased population sizes and decline in immune function, fecundity, and overall species' health. To establish reference data, we measured circulating nutrients (fat-soluble vitamins A and E, four carotenoids, five trace minerals [Cu, Fe, Mn, Pb, Zn], 13 free fatty acids, and 31 amino acids) in 40 HP, 40 GC, and 35 PP from Punta San Juan, Peru. Reference data were established for all parameters. We determined statistically significant differences in nutrient status between species, sexes, and body weight. Peruvian pelicans displayed lower retinol concentrations ( $0.48 \pm 0.07 \mu\text{g/ml}$ ) than other species (GC  $0.67 \pm 0.15 \mu\text{g/ml}$ ; HP  $0.67 \pm 0.13 \mu\text{g/ml}$ ), whereas HP had the highest vitamin E concentrations ( $11.93 \pm 1.57 \mu\text{g/ml}$ ), and lowest of all carotenoids ( $0.04 \pm 0.02 \mu\text{g/ml}$ ) compared with GC ( $5.70 \pm 1.87 \mu\text{g/ml}$ ) and PP ( $10.24 \pm 2.55 \mu\text{g/ml}$ ). Copper concentrations were higher in HP ( $603.98 \pm 123.40 \text{ ng/ml}$ ) than GC ( $352.33 \pm 98.85 \text{ ng/ml}$ ) or PP ( $214.97 \pm 19.81 \text{ ng/ml}$ ). Zinc concentrations were lower in GC than both HP and PP. These results provide reference data on circulating nutrient concentrations to evaluate variation in foraging strategy and prey base that exists across species, as well as temporospatial variation that may result from anthropogenic or ENSO climate-based causes.

**Key words:** Amino acid, fat-soluble vitamins, fatty acid, minerals, nutrition, piscivore

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## DIAGNOSIS AND TREATMENT OF ATOPIC DERMATITIS IN LARGE FLYING FOXES (*Pteropus vampyrus*) AND VARIABLE FLYING FOXES (*Pteropus hypomelanus*) WITH INTRADERMAL SKIN TESTING AND ALLERGY SPECIFIC IMMUNOTHERAPY: A CASE SERIES

Jane Christman,<sup>1\*</sup> DVM, Dunbar Gram,<sup>2</sup> DVM, Dipl ACVD, Jessica Scrivener,<sup>3</sup> Sarah Crevasse,<sup>1</sup> James F.X. Wellehan,<sup>1</sup> DVM, MS, PhD, Dipl ACZM, Dipl ACVM, and Amy Alexander,<sup>1</sup> DVM, Dipl ACZM

<sup>1</sup>University of Florida, College of Veterinary Medicine, Department of Comparative, Diagnostic and Population Medicine, Gainesville, FL 32610 USA; <sup>2</sup>University of Florida, College of Veterinary Medicine, Department of Small Animal Clinical Sciences, Gainesville, FL 32610 USA; <sup>3</sup>Lubee Bat Conservancy, Gainesville, FL 32609 USA

### Abstract

Seasonal atopic dermatitis is an uncommon, yet described condition affecting large flying foxes.<sup>1</sup> This case series describes the management of atopic dermatitis in a captive population of flying foxes (*Pteropus vampyrus* and *Pteropus hypomelanus*) housed at Lubee Bat Conservancy in Gainesville, FL, USA, using intradermal skin testing and allergen specific immunotherapy (ASIT). ASIT has been used in veterinary dermatology for decades for treatment of atopic dermatitis;<sup>2</sup> however, its use is uncommon in zoological medicine. Clinical signs varied between bats, but included restlessness, sneezing, conjunctivitis, and moist ulcerations of non-haired skin, often localized to the carpal regions and toes. Intradermal skin testing was performed under general anesthesia on six bats affected with dermatitis. Allergen specific immunotherapy was formulated specific to each bat and administered subcutaneously or sublingually for a variable duration ranging from 8 mo to over 3 yr. Allergens noted as positive on skin test varied between individuals; however, the majority were allergic to native plant species in the region, including bayberry (*Myrica* sp.), red cedar (*Juniperus virginianus*) and sweet gum (*Liquidambar styraciflua*). No adverse reactions to immunotherapy were recognized in any bat. Reformulation was performed on 3/6 bats due to reoccurrence of clinical signs. Full regression of lesions was noted in 1/6 cases, and all cases showed improvement of clinical signs, degree of skin ulceration, and perceived comfort level.

**Key words:** Allergy testing, atopic dermatitis, flying fox, immunotherapy, *Pteropus hypomelanus*, *Pteropus vampyrus*

### ACKNOWLEDGMENTS

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## AFRICAN PENGUIN POPULATION HEALTH: RESULTS OF THE ASSOCIATION OF ZOOS AND AQUARIUMS REPORTS FROM THE VETERINARY ADVISORS, 2007-2016

*Ellen Bronson, med vet, Dipl ACZM,<sup>1\*</sup> Dawn Zimmerman, MS, DVM,<sup>2</sup> and Samantha J. Sander, DVM, Dipl ACZM<sup>3</sup>*

*<sup>1</sup>The Maryland Zoo in Baltimore, Baltimore, MD 21217 USA; Global Health Program, Smithsonian Conservation Biology Institute, Washington, DC 20008 USA; <sup>3</sup>College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 USA*

### Abstract

The Association of Zoos and Aquariums African penguin Species Survival Plan (SSP) is long-standing and currently includes 50 institutions representing 1063 individual birds. An extensive survey is deployed every 2 yr to gather information regarding the morbidity, mortality, preventive medicine practices, and current research activities across institutions. Over the last 10 yr these reports have yielded a plethora of useful information to aid in the understanding of diseases affecting this commonly-held species and to inform the veterinary advisors of health and research activities within the SSP. Based on the results of the surveys from 2007-2016, the main causes of mortality in this species in order of incidence include aspergillosis, neoplasia, avian malaria, kidney disease, and osteoarthritis. Causes of morbidity include suspected or confirmed aspergillosis, molting issues, avian malaria, arthritis, and pododermatitis. The majority of African penguins (*Spheniscus demersus*) held outdoors in the SSP receive prophylactic antimalarial treatment throughout the mosquito season (typically April through November). In the most recent survey, the most commonly utilized antimalarial drugs were primaquine (24%) or sulfadiazine-pyrimethamine-folic acid (20%) with greatly varying dosing regimens. Preventive health examinations are regularly performed in 78% of surveyed institutions, including vaccination against West Nile virus (WNV). Multiple vaccine reactions of varying severity have been noted after administration of the equine recombinant WNV vaccine. The veterinary advisors recommend malaria prophylaxis for all outdoor colonies in North America due to avian malaria cases reported in this species in all areas of North America over the last 10 yr. Furthermore, vaccination with killed products is recommended for WNV protection.

**Key words:** African penguin, aspergillosis, avian malaria, health survey, *Spheniscus demersus*, veterinary advisor group

### ACKNOWLEDGMENTS

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## INTER- AND INTRA-INDIVIDUAL AGREEMENT OF A SCORING MODEL FOR PODODERMATITIS IN GREATER FLAMINGOS (*Phoenicopterus roseus*)

*Joanna K. Webb, DVM,<sup>1,2\*</sup> Krista A. Keller, DVM, Dipl ACZM,<sup>1,2</sup> Kenneth R. Welle, DVM, DABVP (Avian Practice),<sup>1</sup> Peter Burvenich,<sup>3</sup> Grace Mitchell,<sup>3</sup> Samantha Bradley,<sup>1,2</sup> Eliana Foltin,<sup>1,2</sup> and Matthew C. Allender, MS, DVM, PhD, Dipl ACZM<sup>1,2</sup>*

<sup>1</sup>*Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 USA;* <sup>2</sup>*Wildlife Epidemiology Laboratory, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 USA;* <sup>3</sup>*Miller Park Zoo, Bloomington, IL 61701 USA*

### Abstract

Pododermatitis is a leading cause of morbidity in managed *Phoenicopteridae*. The individual feet of 50 greater flamingos (*Phoenicopterus roseus*) were photographed in fall 2018 while being managed primarily in an outdoor enclosure and in December 2018 after being managed primarily within indoor holding for 1.5 mo. Each randomized image ( $n = 96$ ) was scored based upon a previously described pododermatitis classification rubric that assigns a 0-2 score for hyperkeratosis, fissures, nodules and papillomatous lesions.<sup>1</sup> In addition, a subjective overall pododermatitis score (none, mild, moderate, severe) was assigned. Scoring was performed by 7 blinded individuals: 3 specialists, 2 animal care staff (ACS) and 2 veterinary students. Inter-individual reliability and agreement were evaluated using Krippendorff's alpha and weighted kappa, respectively, to evaluate the scoring model. Randomization of images was repeated, and each image scored again by specialists at least 1 mo later to evaluate intra-individual agreement. Overall, the scoring of fissures exhibited good intra- and inter-individual reliability and agreement; there was reliable agreement between specialists ( $\alpha = 0.782$ ) and between all individuals ( $\alpha = 0.807$ ), good agreement between specialists and ACS ( $\kappa = 0.853$ ) and good or very good intra-individual agreement for all of the specialists. Scoring of the other categories and the subjective score resulted in variable reliability and agreement. Based upon results presented here, it is recommended that modification of the current scoring system be performed to provide a classification scheme that allows for repeated measures of lesions, allowing for assessment of alterations in lesions.

**Key words:** Krippendorff's alpha, *Phoenicopteriformes*, weighted kappa

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## PHARMACOKINETICS AND EFFICACY OF A SINGLE ORAL DOSE OF PONAURIL IN THE INDIAN PEAFOWL (*Pavo cristatus*)

*Stephanie H. Zec, DVM,<sup>1\*</sup> Mark G. Papich, DVM, MS,<sup>2</sup> David A. Oehler,<sup>1,4</sup> Kevin Hils,<sup>1</sup> Susan Schmid,<sup>1</sup> Ken Huth,<sup>1</sup> S. Seville, PhD,<sup>3</sup> and Jean A. Paré, DMV, DVSc, Dipl ACZM, DipECZM (Zoo Health Management)<sup>1</sup>*

<sup>1</sup>Wildlife Conservation Society, Bronx Zoo, Bronx, NY 10460 USA; <sup>2</sup>Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606 USA; <sup>3</sup>Department of Zoology and Physiology, College of Arts and Sciences, University of Wyoming, Casper, WY 82601 USA; <sup>4</sup>Nashville Zoo, Nashville, TN 37211 USA

### Abstract

Apicomplexan protozoa are major causes of morbidity and mortality in avian species. Ponazuril, a novel coccidiocidal triazinetrione, has shown promise in addressing apicomplexan diseases in mammals and birds.<sup>1,5,10</sup> Optimal dosage and dosing intervals in birds remain unknown, and current protocols are extrapolated from studies in mammals.<sup>1,6</sup> This study describes the pharmacokinetics of ponazuril in healthy adult Indian peafowl (*Pavo cristatus*) following a single oral dose administered at two different dosages. Peafowl (four males and four females) were administered ponazuril (Ponazuril, 150 mg/ml, Diamondback Drugs, Scottsdale, AZ 85251, USA) at 20 or 40 mg/kg orally in a double crossover design, with a 2-wk washout period. Coccidial shedding was monitored by serial fecal examination throughout the trials. Serum was collected at 2, 4, 8, 24, 48, 72, 96, and 120 hr after administration. Ponazuril serum levels were analyzed using high performance liquid chromatography. No obvious adverse effects were observed at either dosage, and fecal oocyst shedding subsided in all birds by the end of the study. Ponazuril concentrations peaked at 21.38 hr ± 5.25 for 20 mg/kg and 22.04 hr ± 7.39 for 40 mg/kg with a concentration of 11.82 µg/ml ± 3.01 for 20 mg/kg and 18.42 µg/ml ± 4.13 for 40 mg/kg. Ponazuril was detected at 120 hrs with a concentration of 9.48 µg/ml ± 2.59 for 20 mg/kg and 12.25 µg/ml ± 2.89 for 40 mg/kg. Ponazuril in peafowl is well absorbed orally, concentrations are dose-dependent, and elimination is slower than current dosages for birds would suggest.<sup>3</sup>

**Key words:** Coccidia, Indian peafowl, *Pavo cristatus*, pharmacokinetics, ponazuril

### ACKNOWLEDGMENTS

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## PHARMACOKINETICS AND CLINICAL EFFECTS OF A SINGLE ORAL DOSE OF TRAZODONE IN DOMESTIC GOATS (*Capra hircus*) AS A MODEL FOR WILD RUMINANTS

*Yasmeen Prud'homme, DMV,<sup>1\*</sup> Shannon T. Ferrell, DVM, DABVP (Avian Practice), Dipl ACZM,<sup>2</sup> Sylvain Nichols, DMV, MSc, Dipl ACVS,<sup>1</sup> Francis Beaudry, PhD,<sup>1</sup> and Marion Desmarchelier, DMV, MSc, Dipl ACZM, DipECZM (Zoo Health Management), DACVB<sup>1</sup>*

<sup>1</sup>*Faculté de Médecine Vétérinaire, Université de Montréal, Saint Hyacinthe, QC, J2S 2M2, Canada;* <sup>2</sup>*Société Zoologique de Granby, Granby, QC, J2H 0Y6, Canada*

### Abstract

Trazodone is an anti-anxiety medication commonly used in human and veterinary medicine.<sup>1,3</sup> Stress-related trauma is the leading cause of morbidity and mortality in wild ruminant species. Trazodone could reduce stress and allow safer capture and handling, thus having a positive effect on their welfare. To the authors' knowledge, trazodone use has never been documented in ruminants. The objective of this study was to describe the clinical effects and pharmacokinetic profile of an oral dose of trazodone (Teva-trazodone®, 50 mg tablets, Teva Canada Limited, Toronto, ON, M1B 2K9, Canada) in domestic goats (*Capra hircus*) as a model for wild ruminants. A pilot study using ethograms and actimetry (Actiwatch64, AW64-AM, CamNtech Ltd, Cambridge, CB23 3UY, United Kingdom) identified an oral dose of 10 mg/kg as optimal to reduce activity levels. This dose resulted in a significant increase in time spent sleeping ( $P = 0.0016$ ) and lying down ( $P = 0.008$ ), and decrease in time spent grooming ( $P = 0.023$ ), moving ( $P = 0.005$ ) and observing ( $P = 0.0002$ ). Activity levels were significantly decreased for 4 hr following administration ( $P = 0.049$ ). There were no observed adverse effects. Time spent eating or ruminating was not affected by trazodone administration ( $P > 0.05$ ). The final project evaluated the pharmacokinetics of a single oral dose of 10 mg/kg of trazodone in 7 goats. All animals achieved plasma concentrations over 130 ng/ml, a level considered therapeutic in humans and dogs.<sup>1,2</sup> Mean elimination half-life was  $10.55 \pm 6.80$  hr. All goats achieved  $C_{max}$  in 5-15 min and still had detectable plasma levels at 24 hr. Trazodone appears promising to decrease stress in exotic bovid species. Further research is warranted to establish its efficacy in other ruminant species and clinical situations.

**Key words:** *Capra hircus*, domestic goat, pharmacokinetics, ruminant, sedation, trazodone

### ACKNOWLEDGMENTS

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## CONTROLLED CLINICAL TRIAL USING TERBINAFINE NEBULIZATION TO TREAT WILD LAKE ERIE WATERSNAKES (*Nerodia sipedon insularum*) WITH NATURAL OPHIDIOMYCOSIS

*Ellen Haynes, DVM,<sup>1\*</sup> Matthew C. Allender, DVM, MS, PhD, Dipl ACZM,<sup>1,2</sup> Kristin Stanford, PhD,<sup>3</sup> Kathryn Vivirito, MPH,<sup>1</sup> and Allison Wright, MS<sup>1</sup>*

<sup>1</sup>Wildlife Epidemiology Lab, University of Illinois College of Veterinary Medicine, Urbana, IL 61802 USA; <sup>2</sup>Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign, Champaign, IL 61820 USA; <sup>3</sup>Stone Laboratory, Ohio State University, Put-in-Bay, OH 43456 USA

### Abstract

Ophidiomycosis (snake fungal disease) is an emerging disease caused by the fungus *Ophidiomyces ophiodiicola*.<sup>1</sup> To mitigate the disease's impact on individual snakes, a controlled clinical trial was conducted using terbinafine nebulization<sup>3</sup> to treat snakes with ophidiomycosis. Twenty-five wild-caught Lake Erie watersnakes (*Nerodia sipedon insularum*) with apparent ophidiomycosis (skin lesions present, qPCR<sup>2</sup> positive for *O. ophiodiicola*) were divided into control and treatment groups using matched pairs. Treatment snakes were nebulized with a terbinafine solution (Terbinafine hydrochloride USP 2 mg/ml solution made from grinding up 250 mg tablets, Sigma-Aldrich, St. Louis, MO, and dissolving in 0.9% saline) for 30 min daily for 30 days; control snakes received 0.9% saline. Lesions were tracked weekly and qPCR was repeated after 30 days of treatment. Persistently qPCR-positive snakes received multiple nebulization courses. Terbinafine nebulization reduced fungal quantity by 90.2%, 90.3%, and 98.5% after two, three, and four courses, while fungal quantities in saline-treated snakes were 31.4, 14.7, 8.1, and 10.2 times higher after one, two, three, and four courses. One treatment snake was qPCR negative after two courses, then recrudesced; two others were qPCR negative after three and four courses, without recrudescence. The mortality rates were 75% and 69.2% in the control and treatment groups, respectively. Of the three control and four treatment snakes alive after the fourth course, two showed complete resolution of disease (15.4% of treatment snakes, 0% of control snakes), and five showed persistent disease (15.4% of treatment snakes, 25% of control snakes). These results indicate that, while terbinafine nebulization is a promising treatment for ophidiomycosis, snakes may require multiple nebulization courses and disease may not always resolve completely despite treatment.

**Key words:** Clinical trial, Lake Erie watersnakes, *Nerodia sipedon insularum*, ophidiomycosis, *Ophidiomyces ophiodiicola*, terbinafine

### ACKNOWLEDGMENTS

The authors thank the staff at the Stone Lab for their assistance capturing snakes in the field and the students in the Wildlife Epidemiology Lab at the University of Illinois, College of Veterinary Medicine for their help caring for snakes during the trial.

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## PHARMACOKINETICS OF ORAL MAVACOXIB IN CARIBBEAN FLAMINGOS (*Phoenicopterus ruber ruber*)

Gail L. Huckins, DVM,<sup>1\*</sup> James W. Carpenter, MS, DVM, Dipl ACZM,<sup>1</sup> Sara Dias, MSc, DVM,<sup>1</sup> and Butch KuKanich, DVM, PhD, DACVCP<sup>2</sup>

Departments of <sup>1</sup>Clinical Sciences and <sup>2</sup>Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506 USA

### Abstract

Mavacoxib is a selective COX-2 non-steroidal anti-inflammatory drug that has been used for management of osteoarthritis and other inflammatory conditions in dogs.<sup>1,3</sup> The main advantage of mavacoxib over other non-steroidal anti-inflammatory drugs is its longer plasma half-life, leading to a decrease in dosing frequency.<sup>2,3</sup> This study determined the pharmacokinetics of mavacoxib (Trocoxil<sup>®</sup> 75 mg, Pfizer Italia s.r.l., Ascoli Piceno, Italy) in Caribbean flamingos (*Phoenicopterus ruber ruber*) after a single oral dose of 6 mg/kg ( $n = 6$ ). Blood collection was performed prior to mavacoxib administration and then at 4, 8, 24, 48, 72, 120, 168, and 336 hr after administration. Plasma mavacoxib concentrations were determined using liquid chromatography with mass spectrometry, and pharmacokinetic analysis was performed using noncompartmental methods. Mean peak plasma concentration ( $C_{max}$ ) was (mean; range) 2.97 (2.19-4.06)  $\mu\text{g/ml}$ ; mean time to peak plasma concentration ( $T_{max}$ ) was 18.68 (4.00-48.0) hr; mean area under the curve (AUC) was 455 (292-637)  $\text{hr}\cdot\mu\text{g/ml}$ ; and mean terminal half-life ( $T_{1/2}$ ) was 74.47 (49.57-161.4) hr. Based on the results of this study, mavacoxib dosed at 6 mg/kg orally in Caribbean flamingos reaches plasma concentrations above the therapeutic concentration established for dogs, but further studies are needed to determine appropriate dosing recommendations in flamingos.

**Key words:** Analgesia, Caribbean flamingo, mavacoxib, non-steroidal anti-inflammatory drug, pharmacokinetics, *Phoenicopterus ruber ruber*

### ACKNOWLEDGMENTS

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## TOWARDS AN EVIDENCE-BASED TREATMENT PROTOCOL FOR CERVID BABESIOSIS: PHARMACOKINETICS OF IMIDOCARB IN WHITE-TAILED DEER (*Odocoileus virginianus*)

Ellie L. Milnes, VetMB, DVSc,<sup>1,2,3\*</sup> Pauline Delnatte, DVM, DVSc, Dipl ACZM, DipECZM (Zoo Health Management),<sup>3</sup> Murray Woodbury, MSc, DVM,<sup>4</sup> Ron Johnson, DVM, PhD, Dipl ACVCP,<sup>1</sup> Ronette Gehring, BVSc, MMedVet (Pharm), DACVCP,<sup>5</sup> Dale A. Smith, DVM, DVSc,<sup>1</sup> and Nicole Nemeth, BA, DVM, PhD, DACVP<sup>1,6</sup>

<sup>1</sup>Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada; <sup>2</sup>Toronto Zoo, Toronto, ON M1B 5K7, Canada; <sup>3</sup>Current address: Ol Jogi Wildlife Conservancy, Nanyuki 10400, Kenya; <sup>4</sup>Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4, Canada; <sup>5</sup>Faculty of Veterinary Medicine, Utrecht University, 3584 CL Utrecht, Netherlands; <sup>6</sup>Current address: Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, GA 30602 USA

### Abstract

Cervid babesiosis, caused by infection of erythrocytes with *Babesia odocoilei*, is an emerging tick-borne hemolytic disease of cervids in North America.<sup>1-3</sup> Clinical signs in affected animals include lethargy, respiratory distress, hemoglobinuria, icterus, and sudden death.<sup>1-4</sup> Supportive care for cervids in hemolytic crisis includes anti-inflammatory drugs, fluid therapy to minimize the secondary renal effects of hemolysis, and blood transfusion in very anemic animals.<sup>4</sup> Imidocarb, an anti-protozoal, has been used successfully to treat clinical babesiosis and eliminate parasitemia.<sup>1</sup> This study was designed to investigate the pharmacokinetics of a single dose of imidocarb (3.0 mg/kg i.m; Imizol® 120 mg/ml, Merck Animal Health, Intervet Inc., Madison, NJ 07940, USA) in white-tailed deer (*Odocoileus virginianus*,  $n = 10$ ). Blood samples were collected at 14 time points over a 48-hr period after drug administration, and plasma imidocarb concentrations determined by HPLC with UV detection. The mean  $\pm$  SE maximal imidocarb concentration was  $880.78 \pm 81.12$  ng/ml at  $38.63 \pm 5.30$  min post-injection. The distribution phase had a half-life of  $25.90 \pm 10.21$  min, and plasma imidocarb concentration declined with a terminal elimination half-life of  $464.06 \pm 104.08$  min. The rapid distribution and slow elimination of imidocarb in white-tailed deer following i.m. injection at 3.0 mg/kg may result in plasma concentrations remaining at therapeutic concentrations for at least 8 h. However, pharmacokinetic parameters are not necessarily comparable across species, and caution must be used in generalizing the results of the present study to other cervids. Clinical trials in parasitemic cervids are required to evaluate the efficacy of this dose of imidocarb as a treatment for cervid babesiosis.

**Key words:** *Babesia odocoilei*, cervid babesiosis, imidocarb, *Odocoileus virginianus*, pharmacokinetics, white-tailed deer

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## PHARMACOKINETICS OF A SINGLE ORAL DOSE OF PHENYLBUTAZONE IN SOUTHERN WHITE RHINOCEROS (*Ceratotherium simum simum*)

*Emma L. Houck, DVM,<sup>1\*</sup> Mark G. Papich, DVM, MS, DACVCP,<sup>1</sup> and Katie W. Delk, DVM, Dipl ACZM<sup>2</sup>*

<sup>1</sup>*Department of Clinical Sciences, North Carolina State University College of Veterinary Medicine, Raleigh, NC 27606 USA; <sup>2</sup>North Carolina Zoo, 4401 Zoo Parkway, Asheboro NC 27205 USA*

### Abstract

Southern white rhinoceros (*Ceratotherium simum simum*) in captive and semi-captive settings frequently develop painful conditions, such as traumatic injuries or osteoarthritis, necessitating the administration of pain-relieving medications. A commonly chosen nonsteroidal anti-inflammatory drug (NSAID) is phenylbutazone because of availability of oral formulations and the familiarity of its use in domestic horses.<sup>1</sup> For this study, a single oral dose of phenylbutazone (Phenylbut Powder, 1 g/10 g powder, Phoenix<sup>TM</sup> Pharmaceutical Inc., St. Joseph, MO, USA) at 2 mg/kg was administered to healthy adult white rhinoceros ( $n = 38$ ) housed at six North American zoological institutions. Each rhinoceros had blood samples collected under voluntary behavioral restraint at up to four of the predetermined time points (0, 1, 1.5, 2, 3, 4, 6, 8, 10, 24, 30, and 48 hr). Drug analysis was performed by high pressure liquid chromatography. The pharmacokinetic parameters were calculated with nonlinear mixed-effects modeling using Phoenix<sup>®</sup> software (Phoenix<sup>®</sup>, NLME<sup>TM</sup>, Certara, Princeton, NJ, USA). The preliminary analysis showed a peak concentration ( $C_{MAX}$ ) of 5.64  $\mu\text{g/ml}$  at 2 hr, and a terminal half-life ( $T_{1/2}$ ) of 7.2 hr. The concentrations achieved were similar to what has been reported for horses. The plasma phenylbutazone concentrations were within the effective concentration for 50% response ( $EC_{50}$ ) for horses, which is described as at 1.5 to 4.5  $\mu\text{g/ml}$ .<sup>2</sup> After our successful preliminary study, additional studies are proceeding to examine a large number of treated animals. This study will represent the first pharmacokinetic data of phenylbutazone in any rhinoceros species.

**Key words:** Analgesia, *Ceratotherium simum simum*, pharmacokinetics, phenylbutazone, southern white rhinoceros

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## THE INTERSECTIONS OF GENDER, SALARY AND LIFE: A SURVEY OF AMERICAN COLLEGE OF ZOOLOGICAL MEDICINE DIPLOMATES

*Tara M. Harrison, DVM, MPVM, Dipl ACZM, Dipl ACVPM, DipECZM (Zoo Health Management),<sup>1\*</sup> Kenneth Royal, PhD, MSED,<sup>1</sup> Samantha Morello, DVM, Dipl ACVS,<sup>2</sup> Olivia Petritz, DVM, Dipl ACZM,<sup>1</sup> and Amy Snyder, DVM, MBA<sup>1</sup>*

<sup>1</sup>*North Carolina State University College of Veterinary Medicine, Raleigh, NC 27607 USA;*

<sup>2</sup>*University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI 53706 USA*

### Abstract

It has long been suggested that as a specialty, American College of Zoological Medicine (ACZM) diplomates make lower salaries than other specialists. In 2018, the American Veterinary Medical Association (AVMA) reported that other veterinary boarded specialists made an average of \$129,026 in their first jobs out of their residencies.<sup>2</sup> Our survey of ACZM diplomates in 2018 found that the two most commonly reported salaries for zoo and aquarium veterinarians were \$75,000-\$79,999 and \$90,000-\$94,999 with an average salary of \$90,000. Salaries of ACZM diplomates in academia, on average, were typically \$25,000 or more annually when compared to zoo and aquarium veterinarian salaries. Overall, the most common salaries were \$100,000-\$104,999 and \$110,000-\$114,999. We found a \$15,000 greater difference in overall salaries between females and males, with females making less. As a comparison, the AVMA reported the mean starting salary for new graduate veterinarians was \$76,130 in 2017.<sup>1</sup> We further investigated ACZM diplomate's work-life balance and found that 16% of females delayed having children due to their career, and 64% of the female respondents did not have children at all due to their career (while nearly 65% of the males had children). In addition to choosing to not have children, half of the females reported having children would negatively impact their career. We found other differences in work, life, and family planning between diplomates in our college, indicating that, similar to many other professions, our college is not immune to stressors inherent in our profession.

**Key words:** Children, diplomate, gender, salary, work-life balance

### ACKNOWLEDGMENTS

The authors thank all of the ACZM diplomates who shared their experiences and took the time to complete this survey.

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## THE JUGGLING ACT: ROLE OF ZOO VETERINARY MANAGERS IN CLINICAL PRACTICE

*Lauren L. Howard, DVM Dipl ACZM,<sup>1\*</sup> Michael J. Adkesson, DVM, Dipl ACZM, DipECZM (Zoo Health Management),<sup>2</sup> Gwen E. Myers, DVM,<sup>3</sup> Ellen Bronson, med vet, Dipl ACZM,<sup>4</sup> Ginger L. Sturgeon, DVM,<sup>5</sup> Jennifer D'Agostino, DVM, Dipl ACZM,<sup>6</sup> Allison D. Tuttle, DVM, Dipl ACZM,<sup>7</sup> Meg Sutherland-Smith, DVM, Dipl ACZM,<sup>8</sup> Christopher J. Bonar, VMD, Dipl ACZM,<sup>9</sup> and Donald L. Janssen, DVM, Dipl ACZM<sup>10</sup>*

*<sup>1</sup>San Diego Zoo Safari Park, Escondido, CA 92027 USA; <sup>2</sup>Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513 USA; <sup>3</sup>Zoo Miami, Miami, FL 33177 USA; <sup>4</sup>Maryland Zoo in Baltimore, Baltimore, MD 21217 USA; <sup>5</sup>Pittsburgh Zoo & PPG Aquarium, Pittsburgh, PA 15206 USA; <sup>6</sup>Oklahoma City Zoo and Botanical Garden, Oklahoma City, OK 73111 USA; <sup>7</sup>Mystic Aquarium, Mystic, CT 06355 USA; <sup>8</sup>San Diego Zoo, San Diego, CA 92101 USA; <sup>9</sup>Dallas Zoo, Dallas TX 75203 USA; <sup>10</sup>San Diego Zoo Global (retired), San Diego, CA 92101 USA*

### Abstract

Many zoo veterinarians have upper managerial or supervisory roles while still serving in a clinical capacity. This combination can be challenging due to competing priorities of planned medical procedures, emergent medical cases, managerial responsibilities, and expectations for senior zoo staff level duties.

The purpose of our study was to identify challenges that veterinary managers face in their current roles, and to compare reported priorities among veterinary managers, their direct veterinary reports, and curators. Anonymous, electronic surveys were sent to veterinary managers ( $n = 18$ , 89% response rate), their direct veterinary reports ( $n = 42$ , 48% response rate), and their institution's curators ( $n = 73$ , 38% response rate), representing 18 zoological institutions in North America.

Veterinary managers identified the following activities as being easily manageable: inter-departmental communication, intra-departmental needs, creating value for the department, and administrative tasks. Veterinary managers identified the following activities as being challenging to accomplish: managing clinical cases, finding time to focus on big-picture ideas and personal development.

Interdepartmental communication was in the top one or two priorities identified for veterinary managers by all three work groups. Institutional development was prioritized highly by managers and veterinary direct reports, yet managers acknowledged that they spend little of their time focusing on this task. Conservation projects and/or research/publications were prioritized very low by veterinary managers. Identification of the most common challenges veterinary managers face across institutions, and comparison of the perceptions of the three different working groups, will help align zoo professionals towards common institutional and personal goals.

**Key words:** Communication, curator, management, prioritization, survey, veterinarian

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## **ACKNOWLEDGMENTS**

The authors thank all the veterinarians and curators who participated in this survey, your time and thoughtful comments were very much appreciated! We would also like to thank Adine Nicolson, American Association of Zoo Veterinarians Director of Development, for her work on creating this survey, and reporting the survey results.

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## EVALUATION OF INDUCTION, INTUBATION, AND RECOVERY TIMES WITH FENTANYL OR SUFENTANIL WITH KETAMINE AND MIDAZOLAM IN GORILLAS (*Gorilla gorilla gorilla*)

Ryan S. Bailey, DVM, Dipl ACVAA,<sup>1,2</sup> Julie Balko, VMD Dipl ACVAA,<sup>1,3</sup> and Sathya K. Chinnadurai, DVM, MS, Dipl ACZM, Dipl ACVAA, Dipl ACAW<sup>1\*</sup>

<sup>1</sup>Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513 USA; <sup>2</sup>Premier Veterinary Group, Chicago, IL 60618 USA; <sup>3</sup>North Carolina State University, Raleigh, NC 27607 USA

### Abstract

Due to prevalence of cardiovascular disease in adult gorillas (*Gorilla gorilla gorilla*), recommended anesthetic protocols avoid drugs that can cause significant alterations in cardiovascular dynamics (e.g., alpha-2 agonists) and instead, rely heavily on dissociative anesthetics (e.g., tiletamine).<sup>1-6</sup> While dissociatives like tiletamine are effective anesthetics, the lack of reversibility and long duration of action usually produce prolonged, turbulent anesthetic recoveries.<sup>1,2,6</sup> Fentanyl and sufentanil are both short acting full mu opioid agonists that result in minimal cardiovascular depression in other species, allowing for rapid recovery and cardiovascular stability.<sup>7</sup> This study evaluated coinduction combinations of ketamine and midazolam with fentanyl (FKM) or sufentanil (SKM).

Five male and five female adult gorillas were anesthetized with FKM (fentanyl 1-1.5 µg/kg, ketamine 4-5 mg/kg, and midazolam 0.2-0.3 mg/kg) followed by maintenance with isoflurane or sevoflurane (Table 1). For females, this combination resulted in smooth, calm induction, and calm, rapid recoveries. In male gorillas, multiple supplemental injections of ketamine were required before animals reached a working depth of anesthesia. Based on these results, an additional group of males ( $n = 7$ ) was anesthetized with SKM (midazolam 0.2-0.3 mg/kg, ketamine 4-5 mg/kg, and sufentanil 0.25-0.3 µg/kg) and maintenance with sevoflurane. SKM resulted in smooth inductions and rapid recoveries after reversal with 10 mg naltrexone i.m. per animal. With both FKM and SKM, rare cases of bradycardia were successfully treated with atropine. In FKM females and SKM males, time course from initial injection to intubation was approximately 40 min. Time from reversal to standing in SKM males ranged from 15-54 min (Table 2).

**Key words:** Anesthesia, fentanyl, *Gorilla gorilla gorilla*, induction, ketamine, midazolam

### ACKNOWLEDGMENTS

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**Table 1.** Demographic data and drug dosage amounts, presented as mean  $\pm$  standard deviation, for adult gorillas (*Gorilla gorilla gorilla*) anesthetized with either fentanyl, ketamine and midazolam (FKM) or sufentanil, ketamine and midazolam (SKM).

Treatment group	n	Age (Yr)	Weight (kg)	Fentanyl ( $\mu\text{g}/\text{kg}$ )	Sufentanil ( $\mu\text{g}/\text{kg}$ )	Ketamine (mg/kg)	Midazolam (mg/kg)
FKM female	5	20.26 $\pm$ 7.7	82.8 $\pm$ 10	0.92 $\pm$ 0.12		3.88 $\pm$ 0.47	0.15 $\pm$ 0.01
FKM male	5	25.39 $\pm$ 4.26	182.4 $\pm$ 15	0.79 $\pm$ 0.28		3.93 $\pm$ 0.4	0.14 $\pm$ 0.01
SKM male	7	25.18 $\pm$ 6.0	173.6 $\pm$ 20.7		0.28 $\pm$ 0.05	4.95 $\pm$ 0.44	0.14 $\pm$ 0.02

**Table 2.** Times of anesthetic milestones in minutes from injection, presented as median and range, for adult gorillas (*Gorilla gorilla gorilla*) anesthetized with either fentanyl, ketamine and midazolam (FKM) or sufentanil, ketamine and midazolam (SKM). LMA signifies laryngeal mask airway and ETT signifies endo-tracheal tubes.

Treatment group	First effect	Recumbency	Safe working depth	Time to LMA/ETT placement	Total anesthesia time (time to reversal)	Reversal to sternal	Reversal to standing
FKM female	5.5 (4-8)	12.5 (4-38)	27 (7-40)	42 (20-74)	87 (56-120)	9 (6-13)	10 (8-15)
FKM male	5 (3-11)	23 (6-43)	40 (28-65)	61 (32-76)	158 (72-279)	19 (13-20)	44 (28-81)
SKM male	4.5 (3-15)	22.5 (9-27)	29 (14-43)	39 (17-49)	91 (63-334)	12.5 (3-18)	23 (15-54)

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## REPEATED USE OF A THIAFENTANIL-BASED ANESTHESIA PROTOCOL IN AN OKAPI (*Okapia johnstoni*)

Alexander McFarland, BA,<sup>1\*</sup> Khursheed R. Mama, DVM, Dipl ACVAA,<sup>1</sup> Matthew E. Kinney, DVM, Dipl ACZM,<sup>2</sup> Mary Thurber, DVM, Dipl ACZM,<sup>3</sup> Meredith M. Clancy, DVM, MPH, Dipl ACZM,<sup>2</sup> Nadine Lamberski, DVM, Dipl ACZM, DipECZM (Zoo Health Management),<sup>2</sup> James E. Oosterhuis, DVM,<sup>2</sup> and Lauren L. Howard, DVM, Dipl ACZM<sup>2</sup>

<sup>1</sup>Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523 USA; <sup>2</sup>Harter Veterinary Medical Center, San Diego Zoo Safari Park, Escondido, CA 92027 USA; <sup>3</sup>Oakland Zoo, Oakland, CA 94605 USA

### Abstract

Data evaluating ultra-potent opioids in okapi (*Okapia johnstoni*) are limited, with no documented literature in the species informing thiafentanil use. This report retrospectively summarizes its use from seven anesthetic events performed over a 6-wk period on a 1.5-yr-old female okapi being managed for a fetlock injury. A combination (median; range) of butorphanol (0.045; 0.031-0.046 mg/kg), medetomidine (0.037; 0.031-0.037 mg/kg), ketamine (0.553; 0.536-1.071 mg/kg), and thiafentanil (0.0045; 0.0040-0.0046 mg/kg) (BMKT) was administered intramuscularly with the animal in a padded stall. The first two anesthetic events utilized a single dart for anesthetic delivery, while in all other remaining events, BM was administered approximately 10 min prior to KT using a staggered two-dart protocol. Following initial darting, time (median; range) to first effects, (6; 3-7 min) recumbency, (14; 4-20 min) and restraint (16; 6-22 min) were recorded. Induction quality was subjectively assessed as poor (1), fair (2), good (3) or excellent (4). Median induction quality was superior using the two-dart protocol (4) versus a single dart (1.5). Following recumbency, the okapi was intubated and ventilated. Median (range) anesthesia duration (from initial dart to administration of reversal drugs, which included atipamezole and naltrexone) was 81 min (71-124 min). Neither resedation nor renarcotization were observed, and regurgitation did not occur under anesthesia. Both thiafentanil protocols appear suitable for use in okapi. Similar to existing reported carfentanil protocols, the use of a staggered two-dart protocol provided a qualitatively smoother induction owing to the sedation achieved prior to anesthetic drug administration.

**Key words:** Anesthesia, immobilization, okapi, *Okapia johnstoni*, thiafentanil

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## FIELD ANESTHESIA OF SOUTH AMERICAN SEA LIONS (*Otaria byronia*) IN PERU USING MEDETOMIDINE, MIDAZOLAM, AND BUTORPHANOL

*Michael J. Adkesson, DVM, Dipl ACZM, DipECZM (Zoo Health Management),<sup>1\*</sup> Sathya K. Chinnadurai, DVM, MS, Dipl ACZM, Dipl ACVAA, Dipl ACAW,<sup>1</sup> Julie A. Balko, VMD, Dipl ACVAA,<sup>2</sup> Gwen Jankowski, DVM, MS, Dipl ACZM,<sup>3</sup> Jenny M. Meegan, DVM, Dipl ACZM,<sup>4</sup> Susana Cardenas-Alayza, MSc,<sup>5</sup> and Matthew C. Allender, DVM, MS, PhD, Dipl ACZM<sup>6</sup>*

<sup>1</sup>Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513 USA; <sup>2</sup>Department of Molecular and Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27607 USA; <sup>3</sup>Denver Zoo, Denver, CO 80205 USA; <sup>4</sup>National Marine Mammal Foundation, San Diego, CA 92106 USA; <sup>5</sup>Programa Punta San Juan, Centro para Sostenibilidad Ambiental, Universidad Peruana Cayetano Heredia, Lima, Peru; <sup>6</sup>Wildlife Epidemiology Laboratory, University of Illinois College of Veterinary Medicine, Urbana, IL 61802 USA

### Abstract

Effective conservation program design must include comprehensive information on ecosystem health. As such, conservation efforts at the Punta San Juan (PSJ) marine protected area in Peru include projects assessing population health of the regionally threatened South American sea lion (SASL; *Otaria byronia*). Such evaluation of free-ranging pinnipeds requires careful field anesthesia in close proximity to water, where suboptimal induction characteristics can result in drowning. Since 2011, 62 SASL (39 male [ $174.4 \pm 38.7$  kg], 23 female [ $82.7 \pm 11.3$  kg]) have been safely anesthetized with a fixed ratio combination of medetomidine, midazolam, and butorphanol (MMB,  $0.053 \pm 0.012$  mg/kg,  $0.31 \pm 0.08$  mg/kg, and  $0.31 \pm 0.08$  mg/kg, respectively) delivered intramuscularly via dart. Animals were darted while sleeping alone or in small cohorts (males) and in the middle of the rookery during the peak breeding season (females). Biological sample collection (blood, blubber, tooth, gastric contents, urine, and others), flipper identification tags, and satellite tag placement was possible in all animals. Isoflurane supplementation was used in 13 animals primarily to extend anesthetic duration (started at  $49.4 \pm 18.8$  min for  $19.3 \pm 14.2$  min duration). Animals were intubated and provided with ventilatory support as needed to reduce hypercapnia. Physiologic parameters (HR, RR, temperature, SpO<sub>2</sub>, ETCO<sub>2</sub>) were carefully monitored. Atipamezole ( $0.16 \pm 0.05$  mg/kg), naltrexone ( $0.27 \pm 0.07$  mg/kg), and flumazenil ( $0.0035 \pm 0.004$  mg/kg) were administered as antagonists  $79 \pm 24$  min following darting. Animals displayed coordinated movement  $16 \pm 13$  min following administration. Recoveries were consistently rated as smooth and uneventful.

**Key words:** Anesthesia, anesthetic agents, drugs, immobilization, pinniped, sea lion

### ACKNOWLEDGMENTS

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## COMPARISON OF INHALANT ANESTHETICS IN THE PRAIRIE RATTLESNAKE (*Crotalus viridis*)

Lauren P. Kane, DVM, MS,<sup>1,4\*</sup> Sathya K. Chinnadurai, DVM, MS, Dipl ACZM, Dipl ACVAA, Dipl ACAW,<sup>2</sup> Kathryn Vivirito, MPH,<sup>4</sup> Danielle Strahl-Heldreth, DVM, MS,<sup>3</sup> and Matthew C. Allender, DVM, MS, PhD, Dipl ACZM<sup>4</sup>

<sup>1</sup>Illinois Zoological and Aquatic Animal Residency Program, Urbana, IL 61802 USA; <sup>2</sup>Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513 USA; <sup>3</sup>University of Illinois, College of Veterinary Medicine, Veterinary Teaching Hospital, Urbana, IL 61802 USA; <sup>4</sup>Wildlife Epidemiology Laboratory, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 USA

### Abstract

Inhalant anesthesia is commonly used to restrain reptiles for a range of procedures, but rarely have comparisons between different gases been performed in rattlesnakes.<sup>1,2</sup> Prairie rattlesnakes (*Crotalus viridis*,  $n = 12$ ) were anesthetized using isoflurane, sevoflurane, or desflurane in a randomized cross-over study design to characterize the potency and physiologic effects of each anesthetic gas in the species. Parameters of anesthetic depth were recorded throughout induction and recovery, including loss and return of righting reflex, muscle tone, ability to intubate, response to tactile stimulation (monofilament), and return to spontaneous respiration. Physiologic data were recorded every 5 min throughout the anesthetic procedures, including heart rate, respiratory rate, and percent expired anesthetic gas. Snakes anesthetized with sevoflurane exhibited an anesthetic gas avoidance behavior during induction and had the longest recovery times to extubation ( $P = 0.01601$ ) and return of pressure response ( $P = 0.04575$ ). Snakes anesthetized with isoflurane had the longest righting reflex return ( $P = 0.007488$ ). There was no significant difference between anesthetic gas and loss of pressure response ( $P > 0.05$ ). Snakes anesthetized with desflurane had the quickest loss of righting reflex ( $P = 0.02914$ ) but did not achieve a deep surgical anesthetic plane as 4/12 snakes were unable to be intubated. Sevoflurane and isoflurane provided safe and effective anesthesia, whereas use of desflurane in rattlesnakes, and potentially other vipers, is not recommended.

**Key words:** Anesthesia, *Crotalus viridis*, inhalant, prairie rattlesnake, viper

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**COMPARISON OF SUBCUTANEOUS ADMINISTRATION OF ALFAXALONE-MIDAZOLAM-DEXMEDETOMIDINE AND KETAMINE-MIDAZOLAM-DEXMEDETOMIDINE FOR SEDATION IN JUVENILE BLUE POISON DART FROGS (*Dendrobates tinctorius azureus*)**

*Taylor J. Yaw, DVM, CertAqV, Christoph Mans, Dr med vet, Dipl ACZM, DipECZM (Zoo Health Management), Laura Martinelli, BS,\* and Kurt K. Sladky, MS, DVM Dipl ACZM, DipECZM (Herpetology)*

*Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706 USA*

**Abstract**

Blue poison dart frogs (*Dendrobates tinctorius azureus*) are small, brightly colored frogs, which are commonly maintained in captivity. Due to their small size, sedation or light anesthesia can enhance the quality and safety of physical examinations, diagnostic sample collection, and therapeutic administration. In this study, the sedative effects of subcutaneously administered alfaxalone-midazolam-dexmedetomidine (AMD) (20, 40, 5 mg/kg, respectively) and ketamine-midazolam-dexmedetomidine (KMD) (100, 40, 5 mg/kg, respectively) were compared in a prospective, randomized, blinded, complete crossover study in juvenile blue poison dart frogs ( $n = 10$ ). Both protocols were partially reversed 45 min after administration with subcutaneously administered flumazenil (0.05 mg/kg) and atipamezole (50 mg/kg). Heart rate, pulmonic respiratory rate, various reflexes and behavioral parameters were monitored following drug administration. Both protocols resulted in rapid onset of sedation with loss of righting reflex observed within 5 min for both protocols. Time to complete recovery was similar with both protocols (mean  $\pm$  SD, AMD: 97.5  $\pm$  11.4 min, KMD: 96.5  $\pm$  25.4 min). The AMD protocol resulted in profound pulmonic respiratory depression, while no significant difference in heart rate was found between the two protocols. All frogs were observed eating within 24 hr of sedation. Gastric prolapses occurred in four frogs (AMD: three; KMD: one), which were easily reduced with a cotton-tip applicator and no other adverse reactions were observed. The results of this study provide two different subcutaneous sedation protocols in juvenile blue poison dart frogs.

**Key words:** Amphibian, anesthesia, anuran, blue poison dart frog, *Dendrobates tinctorius azureus*, sedation

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## EVALUATION OF THIAFENTANIL-AZAPERONE FOR ANESTHETIC INDUCTION IN CAPTIVE ADDAX (*Addax nasomaculatus*)

Rebecca M. Eddy, DVM,<sup>1\*</sup> Julie Swenson, DVM, Dipl ACZM,<sup>1</sup> Holly Haefele, DVM,<sup>1</sup> and Natalia Henao-Guerrero, DVM, MS, Dipl ACVAA<sup>2</sup>

<sup>1</sup>Fossil Rim Wildlife Center, Glen Rose, TX 76043 USA; <sup>2</sup>Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA 24061 USA

### Abstract

The efficacy and safety of thiafentanil-azaperone for anesthetic induction in captive addax (*Addax nasomaculatus*) was evaluated. Fifty-seven anesthetic procedures for electroejaculation (EEJ) of seventeen captive addax bulls were performed between May 2018 and February 2019. Addax were darted intramuscularly using thiafentanil ( $0.035 \pm 0.005$  mg/kg) and azaperone ( $0.344 \pm 0.043$  mg/kg). Induction ( $2.7 \pm 1.7$  min) was the time from darting to recumbency. After transportation to the hospital, addax required supplemental ketamine ( $1.613 \pm 0.370$  mg/kg administered i.v.  $7.6 \pm 3.1$  min after recumbency) for intubation, with nine procedures also requiring isoflurane. All animals were anesthetically maintained on isoflurane. Addax were placed in right lateral recumbency for EEJ and sternal recumbency between cycles. Arterial blood samples were collected at 20 and 40 min after darting and analyzed immediately for pH, PaCO<sub>2</sub>, PaO<sub>2</sub>, base excess, bicarbonate, oxygen saturation, and lactate values. Physiologic measurements including heart rate, respiratory rate, temperature, indirect blood pressure, ETCO<sub>2</sub>, and SPO<sub>2</sub> were recorded every 10 min. Thiafentanil was antagonized with naltrexone (administered 25% i.v. and 75% s.c.) at ten times the thiafentanil dose. Recovery time from reversal to standing was  $6.8 \pm 4.4$  min, and recovery was consistently smooth with minimal ataxia. Adverse cardiopulmonary effects observed were hypercapnia (PaCO<sub>2</sub>: T = 20  $51.15 \pm 9.75$  mmHg; T = 40  $51.61 \pm 9.02$  mmHg) and respiratory acidosis (pH: T = 20  $7.341 \pm 0.058$ ; T = 40  $7.339 \pm 0.073$ ). Thiafentanil-azaperone proved to be a safe and suitable combination for anesthetic induction of captive addax, and with supplemental maintenance drugs was appropriate for invasive procedures.

**Key words:** Addax, *Addax nasomaculatus*, anesthesia, arterial blood gases, azaperone, thiafentanil

### ACKNOWLEDGMENTS

The authors thank Allyssa Roberts, LVT, the hoofstock animal care specialists at Fossil Rim Wildlife Center, Dr. Bill Lance, and Wildlife Pharmaceuticals for their assistance with this study.

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## OPERATIONALIZING ONE HEALTH AT AN ASSOCIATION OF ZOOS AND AQUARIUMS INSTITUTION

*Sharon L. Deem, DVM, PhD, Dipl ACZM*

*Saint Louis Zoo Institute for Conservation Medicine, Saint Louis Zoo, Saint Louis, MO 63110 USA*

### Abstract

In 2011, the Saint Louis Zoo launched the Institute for Conservation Medicine (ICM) which takes a holistic approach to wildlife conservation, public health, and sustainable ecosystems to ensure healthy animals and healthy people. Our mission fits within the growing One Health movement—the merging of disciplines to ensure the health of humans, animals and the environments on which all life is dependent.<sup>1,2</sup> During the first 8 yr, the ICM has developed partnerships with veterinary and medical colleges, universities, and a number of NGOs and zoos globally. All programs are directed at addressing wildlife conservation and health challenges, while always keeping an eye on the relationship between the health of wildlife and wild lands, and their influences on public health. We have established roles that zoo vets have within One Health.<sup>3</sup> In addition, we have encouraged messaging to both professionals and the public on why animal and environmental health are important for human health, and how zoos may contribute to One Health.<sup>4,5</sup> The annual One Health Fair, in which veterinary, medical and ecology students form One Health triad teams, working together to solve current conservation and public health challenges, has been hugely successful. These next-gen One Health practitioners are learning a common language across disciplines and then how to present their discoveries to Zoo visitors. In this talk, I share ICM successes and opportunities, along with examples from other zoo One Health programs, to provide suggestions for zoo-based veterinarians to advance One Health both within our institutions and beyond.

**Key words:** Conservation medicine, environmental health, One Health triad, public health

### ACKNOWLEDGMENTS

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## HEALTHCARE TO HEAL THE PLANET: A ONE HEALTH PROOF-OF-CONCEPT IN BORNEO POSITIONED FOR REPLICATION

*Jeff Wyatt, DVM, MPH, DACLAM,<sup>1\*</sup> Andrew Winterborn, DVM, DACLAM,<sup>2</sup> and Kinari Webb, MD<sup>3</sup>*

*<sup>1</sup>Seneca Park Zoo, Rochester, NY 14621 USA; <sup>2</sup>Queens University, Kingston, ON K7L 3N6 CA; <sup>3</sup>Health in Harmony, Portland OR 97214 USA*

### Abstract

An innovative and transformative One Health or Planetary Health initiative in Indonesian Borneo ([www.healthinharmony.org](http://www.healthinharmony.org)), advanced by veterinarians and physicians, provides a 10-yr proof-of-concept well positioned for replication globally. The planetary health concept advocates an interdisciplinary approach to reverse man's unprecedented degradation of the environment, including deforestation and biodiversity loss, as a requisite to further advance human and environmental health globally.<sup>1</sup> When asked, communities totalling 60,000 villagers surrounding 269,000 acres, Gunung Palung National Park in West Kalimantan identified two solutions to stop illegal logging of rainforest if only they had the resources. If provided with training in alternative livelihoods and access to affordable healthcare, they could cease all illegal logging, a livelihood destroying habitat of 2,500 of the world's last orangutans. Veterinarians from Rochester, NY and Kingston, Ontario mentored villager training in smallholder goat husbandry and production. Farmers, recruited from neighboring Java, trained the loggers in compost production and sustainable, organic farming of barren slash and burn plots. Physicians from Yale, Stanford and Rochester set in motion a mentoring program for recently graduated Indonesian physicians in a new rainforest clinic analogous to residency training programs in North American teaching hospitals. The ensuing, high quality and incentivized community health care program administered by Indonesian physicians provides 70% subsidy for patients from non-logging villages. Non-cash payments with manure, compost, organic produce, reforestation seedlings and handicrafts ensure no patient is turned away. The 10-yr metrics of program impact on community and forest health include a stunning 90% decrease in mortality of children under 5 yr of age and an 88% decrease in illegal logging households. Program replication is underway deeper in Borneo in villages adjacent to Bukit Baka Bukit Raya rainforest, twice the size of Gunung Palung, with plans to expand next year in Madagascar. Our proof-of-concept benefiting both human and environmental health illuminates a pathway for planetary health.

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## ZOO NEW ENGLAND'S ONE HEALTH CLINICAL ELECTIVE: INTRODUCING HARVARD MEDICAL STUDENTS TO ONE HEALTH IN A ZOO SETTING

*Eric J. Baitchman, DVM, Dipl ACZM<sup>1\*</sup> and Richard N. Mitchell, MD, PhD<sup>2</sup>*

*<sup>1</sup>Zoo New England, Boston, MA 02121 USA; <sup>2</sup> Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 USA*

### **Abstract**

In a unique collaboration between Zoo New England and Harvard Medical School, MD and MD/PhD students in their senior year have the opportunity to complete a 1-mo clinical rotation where they are encouraged to think about the commonalities of medicine and physiology in health and disease across all species and the context within overall health of the ecosystem. Believed to be the only formal program of its kind, the elective is designed to foster transdisciplinary collaboration to deepen understanding, gain valuable insights, and to benefit healthcare for both humans and animals. The main point behind the experience is to help medical students appreciate the concept of One Health – the intersection of human health, animal health, and ecosystem health. The goal is to understand that humans exist in an ecosystem shared by many other beings and the health of all is interdependent on each other. The One Health concept is not one that is typically taught in medical schools today and exposing students to the broader picture of ecosystem health and the role of biodiversity in protecting human health has an immediate impact on their perception of health in a broader context. The intent is that as these students move through their careers, they see beyond the single organ system they are working on, or the single patient they are treating, and consider all of the patients within the context of the ecosystem they live in, and what they can do to promote the health of all.

Core to our mission at Zoo New England is the preservation of biodiversity and saving species from extinction. Showing medical students how biodiversity is actually beneficial to human health, is an exciting and rewarding experience.

Students accompany the veterinarians in their daily clinical practice and are actively engaged in all aspects of case management, diagnostic work-up, and treatment of zoological species. This foundation of comparative medicine provides the basis for broader discussions on One Health, while a syllabus that includes weekly reading assignments and review of current literature introduces concepts such as the protective role of biodiversity for human health, and how ecosystem disturbances and climate change can influence emerging infectious disease threats.

During the last week of the students' rotation, they are challenged to develop a novel research proposal that benefits both animal and human health and incorporates ecosystem concerns wherever possible. While there is not currently the resource capacity to pursue every avenue of research that the students propose, some have resulted in exciting new partnerships and projects, including the development of genomic investigations of disease in zoological species being conducted between Zoo New England and the Broad Institute of MIT and Harvard. Not only does this work have the potential to improve sustainable management of healthy populations of species, but it also informs a greater understanding of the biological mechanisms of the same diseases in

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humans and has promoted transdisciplinary collaboration between zoo veterinarians, geneticists, and scientists working in human disease research.

The first student to participate in the clinical rotation did so as an independent study in 2015.<sup>1</sup> Word of mouth quickly spread through the medical school and more students followed, leading to faculty interest and the formation of a formal elective. Since the One Health Clinical Elective was formally added to the Harvard Medical School course catalog in early winter 2017, the response has been overwhelmingly positive. It has proven to be an enriching experience for students and Zoo New England staff alike, and has become so popular that there is a substantial waitlist for participation.

Significant national attention was received when a story featuring the program appeared in the July 3, 2018 edition of the New York Times *Science Times* dominating both the front and back covers of the section. Additional media and national television coverage followed.

This is a model that might easily be replicated throughout the country or internationally between other zoos and medical schools. We encourage other zoo veterinarians to consider reaching out to local medical schools and explore similar opportunities. It is extremely gratifying to see how receptive and engaged the students are, and how much they have to offer to the daily clinical practice with our veterinarians. They have elevated our medical knowledge in multiple cases.

Through the elective, we intend that students gain a deeper understanding of how interconnected humans, animals, and the ecosystem truly are, and in turn, think more broadly when confronted with medical challenges. In its most practical form, the zoo rotation is a great way for medical students to understand origins of disease and disease development in species beyond *Homo sapiens*. It's also an opportunity to hone clinical acumen and judgment by working with patients who can't articulate symptoms, and in a setting without the advanced diagnostic and therapeutic resources that typically characterize the Harvard teaching hospitals. However, the One Health initiative represents much more: it is perhaps one of the best ways for students to cerebrally and viscerally understand how life on earth is so interconnected, how the health and diversity of all life on Earth critically impacts human wellness, and how human behaviors affect the rest of the planet.

**Key words:** Animal health, ecosystem health, education, human health, medical students, One Health

#### ACKNOWLEDGMENTS

The authors thank the Animal Health staff at Zoo New England for their dedication to teaching students, especially Dr. Megan Watson, Dr. Brianne Phillips, and our team of veterinary technicians. We also thank Ms. Terry Galuszka, Harvard Medical School registrar, for her assistance with setting up the elective and keeping track of the many students inquiring about registering.

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## RESPONSE AND COLLECTION MANAGEMENT DURING A RABIES OUTBREAK IN FREE-RANGING RACCOONS (*Procyon lotor elucus*) AT ZOO MIAMI

*Marisa Bezjian, DVM,<sup>1\*</sup> Frank Ridgley, DVM,<sup>2</sup> Gabriella Flacke, MVSc, DVM, PhD,<sup>1</sup> James G. Johnson III, DVM, MS, CertAqV, Dipl ACZM,<sup>1</sup> and Gwen Myers, DVM<sup>1</sup>*

<sup>1</sup>*Animal Health Department, Zoo Miami, Miami, FL 33177 USA;* <sup>2</sup>*Conservation and Research Department, Zoo Miami, Miami, FL 33177 USA*

### Abstract

In May 2018, a Florida raccoon (*Procyon lotor elucus*), exhibiting significant aggressive neurologic behavior, was found on Zoo grounds. It was euthanized and submitted to the Bureau of Public Health Laboratory (Miami, FL). It was confirmed rabies positive. Subsequently, Zoo Miami veterinarians reviewed the rabies vaccination status of all mammals in the collection and administered a booster vaccination to any animal that had not received a vaccination within the past year ( $n = 250$ ). After a second positive wildlife case was identified in June 2018, local Department of Health officials began collaborating with the Florida Department of Health, State Public Health Veterinarian, and Zoo Miami staff. Additional rabies cases were confirmed outside zoo grounds: two domestic cats (*Felis catus*) and a North American river otter (*Lontra canadensis*). Health officials enlisted the Conservation and Research Department at Zoo Miami to test the successful delivery of two oral rabies vaccine (ORV) formulations, and to monitor wildlife preference for designing an oral rabies vaccine program.<sup>1</sup> In addition, traps were maintained on zoo grounds by rabies vaccinated Animal Science staff. All unhealthy raccoons were euthanized. Healthy raccoons were heavily sedated for rabies vaccination, distemper vaccination, ivermectin treatment, and right ear notching, and released on zoo grounds.<sup>2</sup> From May 2018 to May 2019, we identified 25 suspect cases with eight confirmed rabies-positive raccoons. In this presentation, we describe this wildlife disease outbreak and management response, which was a One Health partnership among Zoo Miami personnel, Miami public health officials, and the Florida Department of Health.

**Key words:** *Procyon lotor*, rabies, raccoon, wildlife management

### ACKNOWLEDGMENTS

The authors thank the keepers and staff at Zoo Miami for their assistance with the management of our captive collection and surrounding wildlife. We also thank the Administration and Graphics Departments for their efforts to promote signage and public health awareness for zoo visitors.

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## A RETROSPECTIVE STUDY OF BRAIN LESIONS IN CAPTIVE NON-DOMESTIC FELIDS

Alexander R. Viere, BS,<sup>1\*</sup> Andrew C. Cushing, BVSc, Cert AVP (ZM), Dipl ACZM,<sup>1</sup> Edward C. Ramsay, DVM, Dipl ACZM,<sup>1</sup> and Linden E. Craig, DVM, PhD, DACVP<sup>2</sup>

<sup>1</sup>Department of Small Animal Clinical Sciences and <sup>2</sup>Department of Biomedical and Diagnostic Services, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996 USA

### Abstract

This retrospective study identified and characterized brain lesions in captive non-domestic felids from a large cat sanctuary, submitted to the University of Tennessee College of Veterinary Medicine for postmortem analysis. Necropsy reports from January 2002 through December 2018 were examined, and gross images and microscopic slides were reviewed from individual cats where available. In total, 255 cats met the inclusion criteria (brain examined grossly and/or microscopically and age > 30 days), of which 49 cats (19%) were determined to have brain lesions. Eleven different felid species, as well as one captive-bred hybrid (liger) were included in the study, with tigers (*Panthera tigris*) (55%) and lions (*Panthera leo*) (18%) the most common species. Lesions were grouped into six etiologic categories: neoplastic (31%), vascular (23%), inflammatory (22%), congenital (10%), idiopathic (8%), and metabolic (6%). Not included in the brain lesions categorized above were previously undescribed amphophilic globules in the cerebral cortex of many cats with and without other brain lesions. This included 95% of lion and 93% of tiger brains where the cerebral cortex was available for examination. These globules were not associated with clinical disease, and therefore their significance should be interpreted with caution. Overall, brain disease was identified as the primary cause of mortality in 19 of 255 animals (7%), and all 19 had brain lesions. The histopathologic and gross brain changes documented in this study provide insight into specific diseases and pathologic processes that affect the brains of captive large cat populations.

**Key words:** Brain lesion, lion, non-domestic felid, *Panthera*, retrospective, tiger

### ACKNOWLEDGMENTS

The authors thank Ms. Mary Lynn Haven and Ms. Debbie Chaffins at Tiger Haven, as well as Heather Kloft, Mary Catherine Johnson, and the faculty, residents, interns, technicians and lab technicians of the necropsy and zoological medicine services at the University of Tennessee, for their assistance in data acquisition.

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## EXOCRINE PANCREATIC INSUFFICIENCY IN FOUR CAPTIVE TIGERS (*Panthera tigris*)

Lauren M. Mulreany, DVM,<sup>1\*</sup> Edward C. Ramsay, DVM, Dipl ACZM,<sup>1</sup> and Joerg M. Steiner, Dr med vet, PhD, DACVIM (Small Animal Internal Medicine), Dipl ECVIM (Companion Animal)<sup>2</sup>

<sup>1</sup>University of Tennessee College of Veterinary Medicine, Knoxville, TN 37996 USA; <sup>2</sup>Texas A&M University, College Station, TX 77843 USA

### Abstract

Exocrine pancreatic insufficiency (EPI) is a maldigestion syndrome caused by insufficient synthesis and secretion of pancreatic enzymes. This enzyme deficiency leads to the clinical signs of EPI, such as passing of fatty, undigested feces, increased appetite, and a poor haircoat.<sup>2,3</sup> The diagnostic test of choice for EPI in domestic cats is the feline serum trypsin-like immunoreactivity (fTLI). An fTLI of < 8 µg/L is diagnostic for EPI.<sup>1</sup> This case series describes four captive tigers (*Panthera tigris*) from a sanctuary that presented with clinical signs consistent with EPI. Traditional diagnostic tests did not identify an alternate differential diagnosis. Serum fTLI for the diseased tigers were 0.5, 1.2, 1, and 0.9 µg/L (median 0.95 µg/L). Serum from 10 clinically healthy tigers from the same facility were also analyzed to establish some reference values for fTLI, along with serum folate and cobalamin concentrations. Mean fTLI in the healthy tigers was 3.1 µg/L (range 1.9-4.5 µg/L). A Wilcoxon-rank test revealed diseased tigers had significantly lower serum fTLI concentrations than healthy tigers ( $P = 0.0058$ ). Serum folate was significantly greater in the diseased tigers compared to the healthy tigers ( $P = 0.0058$ ). Serum cobalamin concentrations were below the level of detection (< 150 ng/L) in all tigers, both diseased and healthy. All four diseased tigers had improved fecal quality and gained weight with pancreatic enzyme supplementation (PancreaTabs Plus, Henry Schein, Lexington, KY 40511, USA; 8 tablets p.o. per feeding). These cases demonstrate that tigers with clinical signs consistent with EPI and a decreased serum fTLI concentration may have a good clinical response to pancreatic enzyme therapy.

**Key words:** Diarrhea, exocrine pancreatic insufficiency, feline Trypsin-like immunoreactivity, *Panthera tigris*, tiger

### ACKNOWLEDGMENTS

The authors thank the Texas A&M Gastrointestinal Laboratory, Debbie Chaffins, Mary Lynn Haven, and the dedicated staff of Tiger Haven for their assistance in this project.

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## VITAMIN AND MINERAL SUPPLEMENTATION: LAB TRUST, CONTROLS, AND MANAGING EXPECTATIONS FOR CLINICAL RESULTS

*Kathleen E. Sullivan, MS, PhD,\* Shannon Livingston, MSc, and Eduardo V. Valdes, MSc, PhD*

*Disney's Animals, Science, and Environment, Disney's Animal Kingdom, Bay Lake, FL  
32830 USA*

### Abstract

Supplementation of exotic species diets under human care with purified singular micro nutrients often occurs in response to serum values found outside of established reference ranges. While true normal healthy values are not always established for exotic species, literature may exist,<sup>2</sup> or domestic species models are utilized to start comparisons. There are situations where supplementing a deficient nutrient is appropriate, but often a more optimal dietary balance can avoid the need for specific supplementation. A paucity of data exists regarding nutrient bioavailability in varied species, contributing to the potential lack of result when a single nutrient is provided orally to impact serum concentrations; however, while attempting to interpret supplementation, reliability and trust in laboratory analysis is critical. This becomes important based on variations in sample preparation and methodologies used for analysis including LC-MS-MS, HPLC, and ICP-MS.<sup>1,3</sup> Recent studies testing  $\alpha$ -tocopherol in controlled split longitudinal samples of elephant and rhino serum found extreme variation and lack of correlation ( $r = 0.02$ ;  $r = 0.14$ ; respectively) when compared between and within laboratories.<sup>4-6</sup> The Veterinary Laboratory Investigation and Response Network (Vet-LIRN) of the FDA also conducted proficiency evaluations for vitamin E across laboratories, and found challenges with reliability of some laboratories at minimal levels ( $< 0.88 \mu\text{g/dl}$   $\alpha$ -tocopherol; unpublished 2016). Pachyderms and other exotic species with commonly low tocopherol values may warrant further laboratory control testing before supplementation. Basic quality control testing is recommended, including sending blind duplicate samples, repeated split samples across time, or utilizing the National Institute of Standards and Technology controls.

**Key words:**  $\alpha$ -tocopherol, *Ceratotherium simum*, *Diceros bicornis*, laboratory reliability, *Loxodonta africana*, supplementation

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## URINE CONTAMINATION EFFECTS ON URINALYSIS OF ZOOLOGICAL SPECIES

*Catherine M. Bartholf, DVM,<sup>1\*</sup> Debbie Myers, DVM, Dipl ACZM, DipECZM (Zoo Health Management),<sup>2</sup> and Chelsea Sanchez<sup>2</sup>*

<sup>1</sup>*North Carolina State University, College of Veterinary Medicine, Raleigh, NC 27606 USA;*

<sup>2</sup>*San Antonio Zoo, San Antonio, TX 78212 USA*

### Abstract

In zoological collections, sterile urine samples can be difficult to acquire and samples often are obtained from the floor. The aim of this study is to assess how contact time with substrates (hay, soil, mulch), cleaners, floors, feces, and food affect the validity of urinalysis for clinical decision-making. The first phase of the study involved dipstick analysis of sterile water following contact with surfaces and substrates.<sup>2</sup> Sterile water samples were either allowed immediate or extended contact with surfaces or substrates. The second phase of the study involved urinalysis from a commercial laboratory and dipstick analysis of sterile urine samples from an ocelot (*Leopardus pardalis*), a ringtail cat (*Bassariscus astutus*), and two Asian elephants (*Elephas maximus*) following immediate and extended contact with a variety of surfaces and substrates. The study results concluded that both immediate and extended contact with meat and meat juice altered protein, bilirubin, RBC, and WBC results. On dipstick analysis, contact with vegetable mix elevated glucose readings and contact with dirt consistently elevated nitrite levels. On urinalysis, contact with feces increased occult blood, bacteria, protein, and glucose. Overall, contact with wood, metal, concrete, and cleaners had minimal affects on analysis. Microalbumin results were variable in urine samples following contact with surfaces and substrates and require further evaluation along with urine protein creatinine ratios to determine their validity. While this may be the first study to evaluate urine contamination impacts on urinalysis, a number of published accounts demonstrate the importance and significance of urinalysis for patient care.<sup>1</sup>

**Key words:** Urinalysis, urine contamination

### ACKNOWLEDGMENTS

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## METASTATIC MINERALIZATION IN A ZOOLOGICAL COLLECTION OF EARLESS LIZARDS (*Holbrookia lacerata*)

*Sarah A. Cannizzo, MA, VMD, Dipl ACZM,<sup>1\*</sup> Adam Reppert, MS, RD,<sup>1</sup> Ann Ward, MS,<sup>1</sup> Diane Barber,<sup>1</sup> Martha A. Delaney, DVM, PhD, DACVP,<sup>2</sup> and Kimberly L. Rainwater, DVM<sup>1</sup>*

<sup>1</sup>Fort Worth Zoo, Fort Worth, TX 76110 USA; <sup>2</sup>Zoological Pathology Program, Brookfield, IL 60513 USA

### Abstract

Vitamin and mineral supplements are commonly used in diets for zoological and companion animals. Specific nutrient requirements are often unknown, so informed decisions are based on the literature for related species. Starting in November 2017, several cases of metastatic mineralization were detected in a population of earless lizards (*Holbrookia lacerata*) at the Fort Worth Zoo. Since then, 97% (32/33) of the *H. lacerata* population has died with 81% (26/32) of deaths submitted for histopathology. All submitted cases had mineralization in at least one tissue and 77% (20/26) were consistent with metastatic mineralization with no underlying cause detected histologically. The supplement dusted on the food items fed 5-6 times/wk for 2-4 mo was inadvertently switched and the incorrect supplement was found to contain four-fold the intended vitamin D3 concentration, approximately 20,000 IU/kg. Thus, hypervitaminosis D was considered the most likely cause. Interestingly, collared lizards (*Crotaphytus collaris*), also fed prey supplemented 6 times/wk, and 28 other insectivorous species possibly receiving the supplement 2-4 times/wk did not appear affected. During this time, only two other cases of metastatic mineralization were diagnosed in other herpetofauna at this institution. Prior to receiving the incorrect supplement, there were no cases of metastatic mineralization detected in the earless lizard population. These cases highlight species-specific sensitivities and how excess supplementation can have deleterious effects. It is important to confirm inventory at arrival, regularly conduct chemical analysis of supplements, and educate keepers and owners about supplements.

**Key words:** Earless lizard, *Holbrookia lacerata*, hypervitaminosis D, metastatic mineralization, nutritional supplement

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## INVESTIGATION OF THE USE OF BIOMARKERS FOR ANTEMORTEM DIAGNOSIS OF CARDIAC DISEASE IN MARINE MAMMALS

*Melissa J. Joblon, DVM,<sup>1,2\*</sup> Jennifer E. Flower, DVM, MS, Dipl ACZM,<sup>1</sup> Laura A. Thompson, PhD,<sup>3</sup> Kathleen E. Biddle, DVM, DACVP,<sup>4</sup> Deborah A. Burt, BS,<sup>4</sup> Natalie Rouse, MS,<sup>5</sup> Carrie E. Goertz, MS, DVM,<sup>5</sup> Kristen Jetzke, LVT,<sup>6</sup> Richard P. Giovanelli, BS,<sup>4</sup> and Allison D. Tuttle, DVM, Dipl ACZM<sup>1</sup>*

<sup>1</sup>*Department of Animal Care, Mystic Aquarium, a division of Sea Research Foundation, Inc., Mystic, CT 06355 USA;* <sup>2</sup>*Animal Health Department, New England Aquarium, Boston, MA 02110 USA;* <sup>3</sup>*Research Department, Mystic Aquarium, a division of Sea Research Foundation, Inc., Mystic, CT 06355 USA;* <sup>4</sup>*Pfizer, Global Research and Development, Groton, CT 06340 USA;* <sup>5</sup>*Veterinary Services, Sea Life Center, Seward, AK 99664 USA;* <sup>6</sup>*Veterinary Services, Georgia Aquarium, Atlanta, GA 30313 USA*

### Abstract

Cardiac pathology, though extensively documented in marine mammals;<sup>1-5</sup> remains difficult to diagnose ante-mortem. Cardiac troponin I (cTnI) and N-terminal pro-brain natriuretic peptide (proBNP) are used in many species as sensitive and specific biomarkers for a range of cardiac diseases. This study aimed to determine whether cTn and/or proBNP were increased in confirmed cases of cardiac disease in beluga whales (BW) (*Delphinapterus leucas*), sea otters (SO) (*Enhydra lutris*), Steller sea lions (SSL) (*Eumetopias jubatus*), and California sea lions (CSL) (*Zalophus californianus*), compared to controls. Existing immunoassays for cTnI and proBNP were validated utilizing species-specific heart lysate spiked serum. Banked serum samples from animals with confirmed cardiac disease on histopathology (disease: BW  $n = 3$ , SO  $n = 4$ , SSL  $n = 4$ , CSL  $n = 19$ ), and animals with no evidence of cardiac disease (control: BW  $n = 5$ , SO  $n = 2$ , SSL  $n = 6$ , CSL  $n = 6$ ) were analyzed. Serum concentrations of cTnI (ng/ml) and proBNP (pmol/L) ranged as follows: BW: cTnI < 0.03-21.06, proBNP < 200; SO: cTnI < 0.03-0.24, proBNP < 200; SSL: cTnI < 0.03-12.14, proBNP < 200-1,783; CSL: cTnI < 0.03-44.5, proBNP < 200-1,871. Preliminary statistics revealed significantly higher proBNP in diseased CSL (Mann Whitney U test;  $P = 0.017$ ), and a trend toward higher cTnI in diseased SO (Mann Whitney U test;  $P = 0.06$ ). Higher cTnI in diseased animals was seen in other species; however, due to the presence of outliers statistical significance cannot be confirmed. Results suggest that proBNP and cTnI may be clinically useful as ante-mortem diagnostic tools in CSL and SO, respectively. Increasing sample size and further investigation regarding disease severity is warranted and in process.

**Key words:** Beluga, cardiac biomarkers, proBNP, sea lion, sea otter, troponins

### ACKNOWLEDGMENTS

The authors thank the Wild Animal Health Fund (WAHF) for providing generous funding for this project. We would also like to thank Sion Cahoon and Dr. Martin Haulena from Vancouver Aquarium, Barbie Halaska and Dr. Cara Field from The Marine Mammal Center, Natalie Rouse and Dr. Carrie Goertz from Alaska Sea Life Center, and Gayle Sirpenski from Mystic Aquarium for their support and invaluable contributions to this study.

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## ADVANCING IN-HOUSE LABORATORY DIAGNOSTIC CAPACITY: SMALL STEPS IN IMPROVING EFFICIENCY IN ANIMAL CARE AND OPTIMIZED USE OF RESOURCES

*Nicole I. Stacy, DVM, DrMedVet, DACVP\* and Geoffrey W. Pye, BVSc, MSc, Dipl ACZM*

*<sup>1</sup>Disney's Animals, Science, and Environment, Disney's Animal Kingdom, Bay Lake, FL 32830 USA*

### Abstract

In-house laboratories at zoological facilities play key roles in all aspects of sample processing and, depending on availability of equipment and technical skills, sample analysis. While sample processing provides minimum initial measures to ensure adequate sample quality and submission to diagnostic laboratories, the extent of in-house laboratory sample analyses can vary from basic clinicopathologic testing to microbiologic and molecular techniques. The objectives of this presentation are to 1) present three examples for advancing in-house routine laboratory diagnostic capacity requiring minimal capital outlay and to 2) discuss successful outcomes. Three initiatives were chosen: continuing education of our veterinary technician team, using fecal cytology for guidance of additional diagnostic testing of fecal samples, and in-house fibrinogen measurement. Continuing education as a first step contributed to facilitating communication and building upon training sessions. These initial focused special topic seminars were followed by continuous clinical case rounds with discussion of findings relevant to laboratory procedures and problem-solving, leading to noticeable improvements in laboratory work flow. The second initiative was the development of an algorithm on fecal testing using cytologic evaluation in context of clinical findings as a basis for smart decision making, resulting in substantially reduced use of microbiology testing and consequently considerable reductions in cost. The third measure included initiation of in-house fibrinogen testing, which contributed to rapid diagnostic results and additional cost savings. Implementation of these measures are examples of promoting consistency, efficiency, and diagnostic value of in-house diagnostics ultimately contributing to improvements in animal care and optimization of using available resources.

**Key words:** Continuing education, diagnostics, efficiency, in-house laboratory

### ACKNOWLEDGMENTS

The authors thank the dedicated veterinary technician team at Disney's Animal Kingdom and The Seas with Nemo and Friends for their enthusiasm and contributions in all steps of implementation of these efforts.

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## BLOOD COAGULATION ASSESSMENT OF CAPTIVE ASIAN ELEPHANTS (*Elephas maximus*) USING VISCOELASTIC POINT-OF-CARE UNITS

Ryan McCann, DVM,<sup>1\*</sup> Andrew Hanzlicek, DVM, MS, DACVIM (Small Animal Internal Medicine),<sup>2</sup> Michael Wallis, BS,<sup>2</sup> Nicola Di Girolamo, DVM, MSc (EBHC), PhD, DipECZM (Herpetology),<sup>2</sup> Jennifer D'Agostino, DVM, Dipl ACZM,<sup>1</sup> Kay Backues, DVM, Dipl ACZM,<sup>3</sup> Mark Payton, PhD,<sup>2</sup> and João Brandão, LMV, MS, DipECZM (Avian)<sup>2</sup>

<sup>1</sup>Oklahoma City Zoo, Oklahoma City, OK 73111 USA; <sup>2</sup>Department of Veterinary Clinical Sciences, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078 USA; <sup>3</sup>Tulsa Zoo, Tulsa, OK 74115 USA

### Abstract

The leading cause of mortality among juvenile Asian elephants (*Elephas maximus*) under human care is elephant endotheliotropic herpesvirus (EEHV).<sup>1</sup> As the disease progresses, widespread vascular damage, hemorrhage, and potentially death occurs.<sup>2</sup> The purpose of this study was to evaluate the effect of analytic variables (effect of time, activator, repeated pipetting, addition of sodium citrate, and 120 mile transport) when using three viscoelastic point-of-care units to assess Asian elephant blood coagulation. Blood from six healthy (EEHV PCR negative at the time of the study) adult Asian elephants was collected from the ear vein using vacutainers and evaluated at 0.5-, 1-, 2-, 4-, and 24-hr post collection using thromboelastography (TEG<sup>®</sup> 5000 Hemostasis Analyzer System, Haemonetics Corporation, Braintree, MA 02184 USA), rotational thromboelastometry (ROTEM<sup>®</sup> delta, Tem Innovations GmbH, Munich 81828 Germany), and dynamic viscoelastic coagulometry (Sonoclot<sup>®</sup> Analyzer, Sienco, Inc., Boulder, CO 80303 USA). All three units allowed the assessment of coagulation. As time progressed blood became more hypercoagulable, although, the use of new blood tubes for each time point led to less substantial changes up to 4 hr. Repeat pipetting led to significant hypercoagulability. Native fresh blood was hypocoagulable with weaker clot formation when compared to citrated blood at 30 min. Transported blood sample at 24 hr had significant differences when compared with 30 min citrated blood sample. Kaolin and human tissue factor on Sonoclot<sup>®</sup> analyzer produced a narrower range of clotting variables when compared to glass bead, suggesting that these activators might be more clinically useful. Larger scale studies using these methodologies are needed, as well as evaluation of diseased animals. The information provided by this study will allow for methodology optimization for future studies.

**Key words:** Asian elephant, coagulation, dynamic viscoelastic coagulometry, *Elephas maximus*, thromboelastography

### ACKNOWLEDGMENTS

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## PANCREATIC DISEASE IN THE LESSER KUDU (*Tragelaphus imberbis*): A REVIEW OF FOURTEEN CASES

*Michael M. Garner, DVM, DACVP,<sup>1\*</sup> Scott B. Citino, DVM, Dipl ACZM,<sup>2</sup> Wm. Kirk Suedmeyer, DVM, Dipl ACZM<sup>3</sup> and Kimberly L. Rainwater, DVM<sup>4</sup>*

*<sup>1</sup>Northwest ZooPath, Monroe, WA 98296 USA; <sup>2</sup>White Oak Conservation, Yulee, FL 32097 USA; <sup>3</sup>Kansas City Zoo, Kansas City, MO 64132 USA; <sup>4</sup>Fort Worth Zoo, Fort Worth, TX 76110 USA*

### **Abstract**

A review of archival cases from 1995-2018 identified 14/96 lesser kudus (15%) with pancreatic disease. The kudus were from three institutions, and originated from an initial founder stock of 18 animals. Eight affected animals were females and seven were males. Ages at the time of death or euthanasia ranged from 2-132 mo and average age at time of death was 69 mo. All cases had varying degrees of acinar cell depletion, ductular hyperplasia and fibrosis considered severe enough to have caused death or warrant euthanasia. Four had moderate to marked hyperglycemia. An etiologic agent was not identified by histologic or transmission electron microscopic examination. Chronic pancreatic disease is considered an important cause of morbidity and mortality in the lesser kudu. The etiopathogenesis is not understood, but the absence of obvious causes, the occurrence in very young animals, and the lineage of lesser kudus in the United States suggests a genetic basis for this disease.

**Key words:** Lesser kudu, pancreas, pathology, *Tragelaphus imberbis*

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## EPIDEMIOLOGY AND PATHOLOGY OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS FATALITIES IN EUROPE

*Kathryn L. Perrin, BVetMed, Dipl ACZM, DipECZM (Zoo Health Management),<sup>1,2\*</sup> Daniela Denk, DrMedVet, Dipl ECVP,<sup>3</sup> Annemarie T. Kristensen, DVM, DACVIM (Small Animal Internal Medicine), Dipl ECVIM (Companion Animal), Dipl ECVIM (Oncology),<sup>2</sup> Søren Saxmose Nielsen, DVM, PhD,<sup>4</sup> and Mads F. Bertelsen, DVM, DVSc, Dipl ACZM, DipECZM (Zoo Health Management)<sup>1</sup>*

<sup>1</sup>*Center for Zoo and Wild Animal Health, Copenhagen Zoo, Roskildevej 38, 2000 Frederiksberg, Denmark;* <sup>2</sup>*Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrlægevej 16, 1870 Frederiksberg, Denmark;* <sup>3</sup>*International Zoo Veterinary Group, Keighley, BD21 4NQ, UK,* <sup>4</sup>*Section for Animal Welfare and Disease Control, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 8, 1870 Frederiksberg, Denmark*

### Abstract

Elephant endotheliotropic herpesvirus (EEHV) infection is the leading cause of death among captive Asian elephants (*Elephas maximus*). The aim of this study was to use retrospective data and archived histology samples, to identify factors common to EEHV fatalities. Stud book data were collated from all Asian elephants born in Europe during 1985-2017. The risk period for EEHV was defined as 9 mo of age (the youngest known case) to 8 yr. Calves were classified as survivors if they exceeded 8 yr of age by the end of the study period. Fatalities were classified as any calf succumbing to EEHV during the study period. Forty institutions recorded a total of 263 birth events, including still births. Fifty-five calves were either born dead or died in the first 24 hr leaving 208 live calves. Thirty-one of these died in the risk period, only six from non-EEHV confirmed disease, whereas there were 26 EEHV related fatalities identified from 14 institutions, at an average age of 2.7 yr old and with no sex predilection. Nineteen dams (one to three calves each) and 13 sires (one to five calves each) produced calves that succumbed to EEHV. Tissue location and extent of EEHV lesions (hemorrhage, edema, inflammation, inclusion bodies) were assessed on the basis of a histologic review of 22 cases. Findings were compared to EEHV strain, age and duration of clinical signs. The most consistent lesion was myocardial hemorrhage. Results from this study will help generate hypotheses for risk factors leading to EEHV-associated death.

**Key words:** Age, Asian elephant, *Elephas maximus*, pathology, risk factors, sex

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**ELECTROPHORESIS AND ACUTE PHASE PROTEINS IN HEALTHY, INJURED, AND DISEASED SOUTHERN WHITE RHINOCEROS (*Ceratotherium simum simum*) AND BLACK RHINOCEROS (*Diceros bicornis*)**

*Carolyn Cray, PhD,<sup>1\*</sup> Emma J. Hooijberg, BVSc, PhD, Dipl ECVCP,<sup>2</sup> Anne Meyer, DVM,<sup>3</sup> Holly Haefele, DVM,<sup>4</sup> Kimberly L. Rainwater, DVM,<sup>5</sup> and Elizabeth Hammond, DVM Dipl ACZM, DipECZM (Zoo Health Management)<sup>3</sup>*

*<sup>1</sup>Division of Comparative Pathology, Department of Pathology & Laboratory Medicine, University of Miami Miller School of Medicine, Miami, FL 33136 USA; <sup>2</sup>Department of Companion Animal Clinical Studies and Centre for Veterinary Wildlife Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, SOUTH AFRICA; <sup>3</sup>Lion Country Safari, Loxahatchee, FL 33470 USA; <sup>4</sup>Fossil Rim Wildlife Center, Glen Rose, TX 76043 USA; <sup>5</sup>Fort Worth Zoo, Ft. Worth, TX 76110 USA*

**Abstract**

Quantitation of acute phase proteins in nondomesticated mammals by either serum protein electrophoresis or specific acute phase protein assays is becoming more widely available for research and clinical applications.<sup>1,2</sup> Acute phase protein quantitation can be valuable in health assessments as well as for prognostic value.<sup>2</sup> Recent work using agarose gel electrophoresis in serum samples from the southern white rhinoceros (*Ceratotherium simum simum*) defined several significant differences between normal rhinos and animals with wounds related to poaching and other injuries.<sup>3</sup> In an expanded study of the same sample set, 30% of wounded animals were observed to have increased serum amyloid A (SAA) levels determined by a multi-species SAA ELISA (Tridelta, Kildare, Ireland). A working reference interval of 0-20 mg/L was determined. Values in wounded animals ranged from normal levels to an excess of 100 mg/L. A case of suspected leptospirosis demonstrated increased SAA, consistent with changes to the CBC; however, no SAA increases were seen in a separate case of fibromyxosarcoma. The ELISA has been applied to the black rhinoceros (*Diceros bicornis*) under human care and a range of 0-5 mg/L was observed. In a case of long term undetermined illness in a black rhinoceros, the SAA values ranged from 3.8 to 17.9 mg/L. In these and other cases, protein electrophoresis had variable sensitivity in the detection of clinical abnormality or inflammation. The examination of additional samples with varied clinical presentations as well as repeated measures should prove helpful in understanding the clinical utility of SAA quantitation and electrophoresis in rhinoceros under human care.

**Key words:** Black rhinoceros, *Ceratotherium simum simum*, *Diceros bicornis*, protein electrophoresis, serum amyloid A, white rhinoceros

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## DIAGNOSIS AND TREATMENT OF PHEOCHROMOCYTOMA-INDUCED HYPERTENSION AND SYSTOLIC HYPERTENSION SYNDROME IN TWO AFRICAN ELEPHANTS (*Loxodonta africana*)

*Wm. Kirk Suedmeyer, DVM, Dipl ACZM,<sup>1\*</sup> Laura Hatton, DVM, DACVIM (Cardiology),<sup>2</sup> Ryan Bragg, DVM, MS, DACVECC,<sup>2</sup> Brian G. Stockinger, DVM,<sup>1</sup> Rachael Hill, VMIV,<sup>1</sup> and Kacey Solotoff, VMIV<sup>1</sup>*

<sup>1</sup>*Kansas City Zoo Kansas City, MO 64132 USA; <sup>2</sup>Blue Pearl Specialty, Overland Park, KS 66210 USA*

### Abstract

Two African elephants (*Loxodonta africana*) were diagnosed with hypertension through the use of comparative indirect oscillometric blood pressure monitoring (BPs) (Suedmeyer, unpublished data) and clinical signs.

One elephant, a 40-yr-old nulliparous female, collapsed on exhibit then recovered to standing and normal ambulation within minutes. BPs taken for 3 consecutive days and 1 wk later documented extreme elevations in systolic, diastolic, mean arterial pressures, and heart rate. Electrocardiogram (ECG) documented premature ventricular arrhythmias (PVCs). Urinary catecholamine and catecholamine metabolites were diagnostic for pheochromocytoma. Treatment was initiated with prazosin, an alpha-1 adrenoceptor antagonist, and benazepril, an angiotension-converting enzyme inhibitor. Troponin levels were elevated when compared to conspecific controls. Heart rate, respiratory rates, urinary specific gravity, complete blood counts and select serum chemistries, ECGs, and BPs were consistently monitored. A 15% decrease in BPs was noted and elimination of PVCs was achieved within 3 mo. Weekly BPs and bi-monthly ECGs are performed. The elephant remains clinically normal 14 mo after diagnosis. An increase in micturition was noted within 24 hr of prazosin initiation. Hormonal cycling ceased 7 mo after diagnosis and BPs decreased an additional 13%. It is suspected that the pheochromocytoma is influencing androgen and aldosterone secretion resulting in acyclicity and further decrease in BPs.

The second elephant, a 51-yr-old nulliparous female, was diagnosed with systolic hypertension syndrome during routine exams through comparative BPs and clinical signs. Troponin values were undetected. Treatment was initiated with benazepril twice daily. Reduction in BPs were noted within 2 wk and are evaluated weekly.

**Key words:** African elephant (*Loxodonta africana*), benazepril, blood pressure, hypertension, pheochromocytoma, prazosin

### ACKNOWLEDGMENTS

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## LETHARGY, SYSTEMIC INFLAMMATION, AND HYPOPHOSPHATEMIA IN A GROUP OF SOUTHERN WHITE RHINOCEROS (*Ceratotherium simum simum*)

*Anne M. Meyer, DVM\* and Elizabeth E. Hammond, DVM, Dipl ACZM, DipECZM (Zoo Health Management)*

*Lion Country Safari, Loxahatchee, FL 33470 USA*

### Abstract

Hypophosphatemia associated with systemic inflammation has been reported with idiopathic hemorrhagic vasculopathy syndrome in black rhinoceros (*Diceros bicornis*).<sup>1</sup> A similar syndrome has not been reported in southern white rhinoceros (*Ceratotherium simum simum*). During fall 2017, six cases of systemic inflammation and hypophosphatemia of unknown etiology were identified in white rhinoceroses in South Florida. Clinical signs included slow stiff gait, lethargy, and weakness. Two rhinoceroses developed ulcerative lesions on the caudal aspect of the distal front limbs. Histopathology revealed generalized thrombosis, necrotizing vasculitis and eosinophilic cellulitis. Clinicopathologic findings included leukocytosis characterized by neutrophilia ± left shift, neutrophil toxicity, low to normal HCT, mild hypoalbuminemia, mild hyperglobulinemia, and marked hypophosphatemia (0.2-1 mg/dl). Leptospirosis titers to serovar *L. grippityphosa* were positive in 6/6 cases (1:400-1:3200). All six rhinoceroses had been previously vaccinated for leptospirosis, including this serovar, mostly recently in 2014. Convalescent titers were performed in 3/6 cases, though none showed an increasing titer to this serovar. Leptospirosis PCR on urine samples from 3/6 cases was negative. Serum amyloid A was significantly elevated in at least one case and haptoglobin was elevated in 6/6 cases. All cases responded to treatment with enrofloxacin 5 mg/kg p.o., s.i.d and monosodium phosphate supplementation 6 mg elemental phosphorus/kg p.o., s.i.d. Ulcerative vasculitis lesions were treated with topical antimicrobials and steroids and eventually healed with no other lesion development. No recurrence of these episodes has been seen. Although a similar syndrome has been previously described in black rhinoceroses, this is the first report of this clinical syndrome in southern white rhinoceroses.

**Key words:** *Ceratotherium simum simum*, hypophosphatemia, leptospirosis, southern white rhinoceros, vasculitis

### ACKNOWLEDGMENTS

The authors thank the training and rhinoceros keeper staff at Lion Country Safari for their assistance in sample collection and the care of these animals. The authors thank Dr. Carolyn Cray for her assistance with the serum inflammatory markers.

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## UPDATE FROM THE NORTH AMERICAN ELEPHANT ENDOTHELIO TROPIC HERPESVIRUS (EEHV) ADVISORY GROUP AND SUMMARY OF THE 2019 EEHV WORKSHOP

*Lauren L. Howard, DVM, Dipl ACZM<sup>1\*</sup> and Christine M. Molter, DVM, Dipl ACZM<sup>2</sup>*

*<sup>1</sup>San Diego Zoo Safari Park, Escondido, CA 92027 USA; <sup>2</sup>Houston Zoo, Houston, TX 77030 USA*

### **Abstract**

In March 2019, the Houston Zoo and Elephant Endotheliotropic Herpesvirus (EEHV) Advisory Group hosted the North American EEHV Workshop, which included > 100 in-person and online attendees from North America, Europe, Asia, and Australia. More than 30 lectures covered all aspects of EEHV, including advances in serologic detection of EEHV1A exposure; early steps in vaccine development; reports from the United States, Canada, Myanmar, and India; and the importance of EEHV training benchmarks in calves. In addition, an EEHV preparedness panel, clinical pathology laboratory, husbandry demonstrations, and EEHV practice drill were held. Very recent experiences with fatal EEHV hemorrhagic disease cases in African elephants also were shared with the group, leading to further discussion on how to approach EEHV monitoring and preparedness in African herds.

The EEHV Advisory Group was formed in North America in 2013. The advisory group's mission is to decrease elephant deaths due to EEHV while supporting elephant holding institution programming by 1) disseminating knowledge of current best practices for prevention, diagnosis, and treatment of EEHV; 2) providing private and public elephant-holding facilities with technical assistance; and 3) facilitating research by building international collaborations. The advisory group is a subset of the AZA Elephant Taxon Advisory Group and members are comprised of veterinarians, researchers, elephant management specialists, media specialists, and conservationists.

At the biennial EEHV Advisory Group meeting held at White Oak Conservation Center in August 2018, the advisory group was re-structured to better meet the needs of elephant-holding institutions. In addition to the existing steering committee, four subcommittees were formed, each with a committee chair. The outreach committee assists with updating the [www.eehvinfo.org](http://www.eehvinfo.org) website, provides speaking points, and gives assistance to institutions with EEHV-related questions or needs. The research committee manages requests for support letters, maintains an EEHV-related research data-base, and the advisory group's EEHV-related research priority list. The Veterinary and Management Resources Committee maintains and updates treatment, training, and necropsy recommendations. The Scientific Program Committee identifies hosts for the biennial North American EEHV Workshop and develops the scientific program, in conjunction with the host institution, as well as ensuring EEHV-focused representation at other key meetings. International liaisons from Europe, Asia, and Africa also were identified.

**Key words:** Advisory group, AZA, EEHV, elephant endotheliotropic herpesvirus, *Elephas maximus*, workshop

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## FINANCIAL HEALTH FOR THE ZOO VETERINARIAN

*Travis Hornsby, CFA and Rob Bertman, CFA, CFP®*

*Student Loan Planner, 20 S Sarah St, Saint Louis, MO 63108 USA*

### Abstract

#### **Part I. Veterinary student debt: How did debt burdens get so huge?**

- Grad PLUS program and how it allowed schools to charge unlimited amounts
- Cliche that “student debt is good debt”
- Change in the way Congress sets interest rates

#### **Part II. Repayment plans: What are they and why do they matter?**

- PAYE, REPAYE, IBR
- Tax filing status with your spouse and how it impacts your payment
- Forgiveness vs refinancing: what you should know

#### **Part III. Loan forgiveness programs that zoo veterinarians can utilize.**

- PSLF
- 20 to 25 year IDR forgiveness
- Other programs

#### **Part IV. The most important statistic in your financial life is not your debt.**

- When can you afford retirement?
- How important is it to choose the right loan strategy?
- What action steps do you need to take right now to make sure you’re financially secure?

#### **Part V. Q&A, if time allows.**

Audience participation.

**Key words:** Debt, financial health, loan

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## GERIATRIC ELEPHANT SURVEY OF MEDICAL CARE, NUTRITION, HUSBANDRY, AND WELFARE: INITIAL RESULTS

*Whitney Greene, DVM<sup>1\*</sup> and Deena Brenner, Dipl ACZM<sup>2</sup>*

*<sup>1</sup>Mote Marine Laboratory and Aquarium, Sarasota, FL 34236 USA; <sup>2</sup>San Diego Zoo Global, San Diego, CA 92101 USA*

### Abstract

As medical advances, husbandry and welfare continue to improve in zoos and aquariums, animals are often living longer and experiencing age-related diseases.<sup>1,2,4</sup> Geriatric diseases in zoo animals may be challenging to manage based on variables such as species, social needs, resiliency and compliance. Veterinary hospice and palliative care are hindered by an inadequate amount of scholarly research to guide clinicians;<sup>1,3</sup> this is especially true for zoo and exotic species.<sup>2</sup> Providing optimal welfare for an animal relies on a strong preventive medicine program, appropriate nutrition, husbandry and medical care. This can be especially challenging in geriatric animals. The goal of this study was to conduct a survey to gather information pertaining to geriatric elephant medicine, management, husbandry, and nutrition. An electronic survey was created and sent out to AAZV and EAZWV members through an online link. There were 61 responses which included veterinarians, nutritionists, and elephant managers with a total of 314 elephants in their care, 142 geriatric (over 40 yr old) of which 45 were known to be on their final set of molars. The most common diseases reported were osteoarthritis, foot disease, and colic. Flunixin meglumine and phenylbutazone were the analgesics reported with the highest frequency. Respondents described unique nutritional modifications made to accommodate animals with dental attrition and implementation of a variety of integrative medicine modalities. The initial results of this study provide information that may be applied to improve the welfare of geriatric elephants. Having an understanding of common physical, cognitive, and behavioral changes associated with aging can help better inform management practices and improve overall welfare.<sup>3</sup>

**Key words:** African elephant, animal welfare, Asian elephant, *Elephas maximus*, geriatric medicine, gerontology, *Loxodonta africana*

### ACKNOWLEDGMENTS

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## CHANGES IN ZOO VISITOR PERCEPTIONS OF VETERINARY CARE AND WELFARE FOLLOWING VIEWING OF MEDIA STORIES ON ZOO ANIMAL HEALTH

*Michael J. Adkesson, DVM, Dipl ACZM, DipECZM (Zoo Health Management),<sup>1\*</sup> Gloria Sari, BS,<sup>2</sup> and Yvonne Wong, BS<sup>2</sup>*

*<sup>1</sup>Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513 USA; <sup>2</sup>University of Illinois, College of Veterinary Medicine, Urbana, IL 61802 USA*

### Abstract

Zoo animal health stories are increasingly common in the media, with institutions recognizing that these behind-the-scenes public relations opportunities highlight the quality of animal care. Previous research has shown that visitors are more likely to have positive impressions of zoological parks and take conservation actions if they speak with animal care staff or have up close animal encounters.<sup>1,2</sup> We hypothesized that video media related to veterinary care in a zoo would, (1) educate visitors and (2) positively increase their opinions of zoo animal care and welfare. Zoo visitors (n = 127) were randomly selected to complete a survey, watch one of five different video segments (2-6 min duration) about veterinary care of animals at Brookfield Zoo (BFZ), and then answer an identical set of questions. Video segments focusing on advanced veterinary care for rhinoceroses, lions, and orangutans were produced by BFZ and Chicago evening news programs. Survey questions used a sliding seven-point scale. Across all videos, 11/13 questions revealed significant positive change ( $P < 0.05$ ) in visitor perceptions. Ratings of veterinary care ( $P < 0.0001$ ), animal welfare ( $P < 0.0001$ ), the role of zoos in conservation ( $P = 0.0002$ ), and visit satisfaction ( $P = 0.0187$ ) all improved after video viewing. A majority of respondents (78.0% initial, 89.7% post-video) overestimated how many veterinarians were employed at BFZ and only 46.5% of respondents were able to estimate the collection size within 1,000 animals. Media stories about zoo veterinary care may represent a powerful tool to engage the public, improve perceptions of animal care and welfare, and share important conservation messages.

**Key words:** Animal welfare, media, public relations, television, zoo veterinarian

### ACKNOWLEDGMENTS

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## PRACTICAL TOOLS TO ASSIST WITH AUDITING WELFARE IN ZOOS

*Sarah Chapman, BVM&S, MSc, Dipl ZooMed (Mammalian), MRCVS<sup>1\*</sup> and Sabrina Brando, BSc, MSc<sup>2</sup>*

*<sup>1</sup>Chapman Zoo Consultancy, England; <sup>2</sup>AnimalConcepts, University of Stirling, Scotland*

### Abstract

Welfare assessment in zoos is challenging and involves the use of a combination of measures and approaches to assess an individual's quality of life.<sup>5,6</sup> These can include visual or hands-off methods; inspection of health, husbandry and demographic records and the use of scientific studies. Behavioral indicators and physiologic measures can be combined with clinical and pathologic reviews as part of an overall assessment with additional information about enclosures and husbandry.<sup>2</sup> Many of these processes can be time consuming and rely heavily on the standard of records kept. Additional non-invasive measures of health require access to samples, such as urine and faeces. These can be challenging to collect and store, require laboratory access and the necessary budget for diagnostic testing. Many large collections have research departments and access to staff and students for data collection and entry, whereas smaller zoos or those in less developed areas, do not have these options.

There have been a number of recent publications which have reviewed techniques for monitoring welfare.<sup>1,3,6</sup> The Zoos Expert Committee in the United Kingdom produced a summary of systems used to assist zoos with this process.<sup>2</sup> It is accepted that for welfare to be monitored, longitudinal data is required and robust methodology needed. Welfare auditing tools can be used to assist with this process<sup>4</sup>. This presentation will outline the use of two different tools which have been used as part of an auditing process in a number of different zoological collections internationally.

One tool involved the use of a checklist of questions which assessed the following aspects of the animals' lives (i.e., resource-based measures):

- Physical environment
- Social environment
- Nutrition
- Behavior
- Health and animal care

A total of 25 questions were asked per enclosure/species. Questions in each section were given a score of 0 (not met), 1 (partly met) or 2 (met). A maximum score of 50 was available. The majority of the questions could be answered by talking to the keepers and viewing the animals and enclosures. Others required more time (e.g., behavioral assessment, clinical and pathology reviews). The scores were then placed in a table to create a priority list for the zoos to be able to work on improvements and highlight areas of concern. The check list could then be repeated to monitor scores over time or after significant changes had occurred within the zoo.

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The second tool used the World Association of Zoos and Aquariums Welfare Strategy 2015 which has nine chapters and is based on the Five Domains model of animal welfare.<sup>7</sup> This was used to assess welfare efforts at zoological collections. Each chapter has a list of recommendations and questions. These lists were discussed with the senior management of zoological collections and details filled into a table. Site visits, including viewing behind the scenes, and discussions with keepers supported details to be added to the table. Each row was then assigned a color. Green indicated that processes and procedures are in place, are monitored, updated and reviewed as appropriate. Amber indicated that processes and procedures are partially in place and red indicated that no processes or procedures are in place. These color assignments were then used to create a priority list for improvements. This list can be used as the basis of an action plan for follow up with deadlines allocated as appropriate.

These tools were effective and practical to use in a variety of captive animal situations. They are also easily repeatable to monitor changes over time. They can be used to highlight concerns and focus further evaluation of welfare at an individual level.

**Key words:** Assessment, audit, tools, welfare, zoo

#### **ACKNOWLEDGMENTS**

The authors thank all the collections where these tools were used and helped to highlight priority areas which lead to improvements in animal's welfare.

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## WELFARE CONSIDERATIONS DURING COORDINATION OF MULTI-ORGANIZATIONAL FIELD CONFISCATIONS OF LARGE CARNIVORES AND EXOTIC ANIMALS

*Wm. Kirk Suedmeyer, DVM, Dipl ACZM*

*Kansas City Zoo, Kansas City, MO 64132 USA*

### **Abstract**

The Kansas City Zoo considers itself a community resource and lends its expertise to area endeavors. In particular, the zoo has recently participated in three separate confiscations involving 10 carnivores, including a Bengal tiger (*Panthera tigris tigris*), mountain lions (*Felis concolor*), a black bear (*Ursus americanus*), lynx (*Lynx canadensis*), bobcats (*Lynx rufus*), and a serval (*Leptailurus serval*). Additional confiscations involved a chimpanzee (*Pan troglodytes*) and a Bennetts wallaby (*Macropus rufogriseus*) all housed in either substandard or inhumane facilities.

Coordination and planning involved human organizations, sanctuaries, local and state regulatory officials and media representatives. Animals were either netted, manually restrained or immobilized. Health assessments, performed on all animals, included physical exam, ophthalmic exam and tonometry, indirect oscillometric blood pressures, thermography, pulse oximetry, complete blood counts, select sera chemistries, viral and bacterial serology, and rectal swabs for enteric flora identification and sensitivities. In addition, vaccination, prophylactic deworming, microchip identification, letters of veterinary transport, health certificates and regulatory statements were provided.

Unique challenges are presented when attempting to immobilize animals that have been recently fed, inadequately housed and have questionable health histories. Proper planning for inclement weather will influence success of the immobilization because it impacts both personnel and animal comfort.

The legal aspects to be aware of an address include who owns the animal and takes responsibility for actions of the zoo/participants, approval to participate in and transfer of exotic animals across state lines, permission and support of the institution, safety of personnel and private individuals, firearms usage, veterinary instruction for post-operative care and conveyance of health status of individual animals. The state veterinarian and local regulatory officials are key contacts for assistance in this regard. In some cases, individual owners complicate procedures, therefore local law enforcement personnel should accompany any confiscation.

The coordination of large-scale confiscations presents significant and unique logistical, political, welfare and medical challenges for the zoo veterinarian. In these cases, institutional financial support reduced the need for compensatory funding from owners or regulatory agencies. Close communication with all parties involved is integral to success. Zoo veterinarians should be aware of the potential for involvement with confiscations and legal ramifications of participation in such procedures. Preparation and communication is key to the success and welfare of the animals.

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**Key words:** Carnivore, confiscation, primate, regulatory, welfare

**ACKNOWLEDGMENTS**

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## USE OF A PRESSURE MAT FOR QUALITY-OF-LIFE ASSESSMENTS ON A GERIATRIC RETICULATED GIRAFFE (*Giraffa camelopardalis reticulata*)

Hilary Householder, DVM,<sup>1\*</sup> Sarah Muirhead,<sup>2</sup> Doug Russo, CJF, AWCF,<sup>2</sup> Valerie Johnson, DVM, MS,<sup>3</sup> and June Olds, DVM<sup>1,2</sup>

<sup>1</sup>Blank Park Zoo, Des Moines, IA 50315 USA; <sup>2</sup>Department of Veterinary Clinical Science, Iowa State University, Ames, IA 50011 USA; <sup>3</sup>Department of Clinical Sciences, College of Veterinary Medicine & Biomedical Sciences, Colorado State University, Fort Collins, CO 80523 USA

### Abstract

Lameness is one of the most common problems plaguing adult giraffe in human care and is frequently a factor resulting in humane euthanasia.<sup>1</sup> Lameness may be secondary to a variety of causes, including osteoarthritis, hoof overgrowth, fractures, or systemic diseases caused by metabolic disease or nutritional deficiencies.<sup>1</sup> Common diagnostic procedures include visual examinations with subjective lameness scores, thermography, and radiology. A 21-yr-old female reticulated giraffe (*Giraffa camelopardalis reticulata*) was placed on quality of life monitoring due to a chronic history of malunion fractures of P2 of the lateral claw and of P3 of the medial claw of the right front foot causing chronic, progressive lameness. Using behavioral training, gait analysis was performed using a Tekscan pressure mat to obtain objective data before and after an anesthetized examination with multiple treatments performed. Utilizing the pressure mat, initial gait analysis indicated reduced vertical force placed on the right front foot (109.85 kg) compared to the left front foot (145.18 kg), as well lower maximum peak pressure applied to the right front foot (160 Kpa) versus the left (168 Kpa). Serial monitoring with the pressure mat showed right front foot improvement with 77% increase in vertical force applied and 118% increase in maximum peak pressure applied. This case demonstrates a relatively easy, effective, and objective method to assess giraffe lameness using force plate analysis that provides useful unbiased information aiding in quality-of-life decisions.

**Key words:** *Giraffa camelopardalis reticulata*, lameness, pressure mat, quality-of-life assessment, reticulated giraffe, Tekscan

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## OPTIMIZING RAPTOR WELFARE FROM ACQUISITION TO DISPOSITION

*Michelle Willette, DVM, MPH, Dipl ACVPM,\* Julia Ponder, DVM, MPH, Lori Arent, BS, MS, Gail Buhl, BS, and Kelsey Griffin, BS*

*The Raptor Center, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108 USA*

### Abstract

Raptors are extremely popular as outreach ambassadors and exhibit animals. Birds are generally obtained through wildlife rehabilitation programs (Willette, unpublished data) or captive-bred ( $\pm$  imprinted).<sup>3</sup> There are programmatic and welfare implications for birds from either source: raptors obtained from wildlife rehabilitation facilities are permanently impaired; raptors imprinted on humans often show malfunctional behavior, including aggression.<sup>4</sup> Veterinarians play a vital role in the acquisition of these birds and their ensuing health and welfare.

The Association of Zoos and Aquarium's definition of animal welfare refers to an animal's collective physical, mental, and emotional states over a period of time, and is measured on a continuum from good to poor.<sup>2</sup> There are a variety of welfare assessment tools available;<sup>7</sup> the animal-centric measurements are used to approximate the animal's quality of life (QoL). Zoological medicine has moved beyond health related QoL to overall QoL, establishing baselines and benchmarks for individual animals in advance of any health issues.<sup>8,9</sup> Establishing individualized QoL baselines is critical for these long-lived, special needs birds.

As part of the Partners-4-Wildlife welfare initiative,<sup>6</sup> The Raptor Center has developed a suite of tools to improve the welfare of captive raptors. These tools aid with optimizing the outcome of raptors admitted for rehabilitation,<sup>1,5</sup> considerations for captive placement, and a process for monitoring QoL in captive raptors utilizing health metrics, ethograms, and activity budgets. Recently the tools have been utilized for placement and end-of-life decisions, assessing response to pain medications and in resolving regulatory issues.

**Key words:** Ambassador animals, imprint, quality of life, raptor, welfare, welfare assessments

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## RELIEVING SILENT SUFFERING: MANAGEMENT OF DENTAL CONDITIONS IN ZOO ANIMALS

*Jamie A. Berning, DVM<sup>1\*</sup> and James G. Johnson III, DVM, MS, CertAqV, Dipl ACZM<sup>2</sup>*

*<sup>1</sup>Midwest Mobile Veterinary Dentistry, Dublin, OH 43017 USA; <sup>2</sup>Department of Animal Health, Zoo Miami, Miami, FL 33177 USA*

### Abstract

Dental disease is common in veterinary patients and often under-diagnosed, including in zoo animals where oral evaluation is often limited to opportunistic clinical examination.<sup>1</sup> Underlying oral disease can be present with subtle clinical signs that may not be readily apparent to animal care professionals and there is profound variation in dental anatomy among zoo species.<sup>2</sup> Four cases from two zoological institutions are used to illustrate animals with marked dental disease despite vague clinical signs and where advanced dental intervention resulted in good welfare outcomes. These include a spotted hyena (*Crocuta crocuta*) with a 1-yr clinical history of not fully consuming bones and where previous endodontic therapy of the right mandibular canine tooth and left maxillary fourth premolar tooth failed, necessitating endodontic revision and extraction. An Alaskan brown bear (*Ursus arctos*) presented only for allowing his conspecific to persistently lick the inside of his mouth, but had no change in appetite or diet preferences. The bear had multiple fractured and nonvital teeth that required endodontic and exodontic treatment. A Brazilian porcupine (*Coendou prehensilis*) presented with clinical signs of choke, and further evaluation revealed a periodontally compromised left maxillary third molar tooth and left mandibular second and third molar teeth that were extracted resulting in resolution of clinical signs. Lastly, an African lion (*Panthera leo*) presented for facial swelling and hemorrhagic ocular discharge caused by fungal oral disease that required exodontia and medical therapy. Thorough evaluation during preventive health examination and recognition of obscure clinical signs is essential to early diagnosis and management of dental conditions that may cause unnoticed pain and compromised quality of life.

**Key words:** Dentistry, endodontic, exodontia, oral disease, periodontal, zoo animal

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## FATAL NONREGENERATIVE ANEMIA IN RETROVIRAL-INFECTED KOALAS (*Phascolarctos cinereus*): A POSSIBLE SEQUELA WITH CHARACTERISTIC CLINICAL MANIFESTATION

*Matthew Golembeski, DVM,<sup>1\*</sup> Nicole I. Stacy, DVM, DrMedVet, DACVP,<sup>2</sup> Bruce Rideout, DVM, PhD, DACVP,<sup>3</sup> and Cora Singleton, DVM, Dipl ACZM<sup>4</sup>*

<sup>1</sup>*Michigan State University, College of Veterinary Medicine, East Lansing, MI 48824 USA;*  
<sup>2</sup>*Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL 32608 USA;* <sup>3</sup>*Disease Investigations, Institute for Conservation Research, San Diego Zoo Global, San Diego, CA 92112 USA;* <sup>4</sup>*San Diego Zoo, San Diego Zoo Global, San Diego, CA 92101 USA*

### Abstract

Retroviral endogenization of the koala genome has been ongoing since the 19<sup>th</sup> century.<sup>1</sup> Koala retrovirus (KoRV) genotypes (KoRV-B, KoRV-J) distinct from the originally described Koala Retrovirus A (KoRV-A) have been discovered recently.<sup>2</sup> Retroviral infection in koalas have been associated with significant morbidity and mortality, including leukemia, lymphoma, myelodysplasia, and immunosuppression leading to opportunistic infections, particularly with *Chlamydia* sp. or *Cryptococcus* sp.<sup>3</sup> One important sequela of some retroviral-infected koalas is myelodysplasia, specifically ineffective hematopoiesis with consequent peripheral cytopenias, often leading to severe nonregenerative anemia.<sup>4,5</sup> Myelodysplasia may be a direct or indirect result of retroviral infection.<sup>5</sup> This case series presents a description of clinical signs, duration and severity of illness, and treatment attempts of six KoRV-infected koalas with fatal anemia. Age at presentation ranged 0.6-12.8 yr (average 5.8 yr). Packed cell volume at initial presentation ranged 3-25% (average 17%). Average time from presentation to death was 38 days (range 10-82 days). Treatments with erythropoietin and/or blood transfusion were unsuccessful. Mortality in each case was attributed to the effects of bone marrow dyscrasia and consequent severe nonregenerative anemia. Although koalas presented in this case series either tested positive for or were presumed positive for KoRV-A, a causative relationship between retroviral infection and development of nonregenerative anemia has not been definitively verified to date. Early diagnosis of this hematologic manifestation is obtainable through blood film review and concurrent cytologic examination of bone marrow aspirates<sup>5</sup> both of which provide guidance for clinical case management decisions and should be considered an essential part of koala preventive medicine examinations.

**Key words:** Erythropoiesis, koala, myelodysplasia, *Phascolarctos cinereus*, retrovirus

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## EXPLORATION OF VITAMIN D METABOLISM IN INDOOR-HOUSED HOFFMANN'S TWO-TOED SLOTHS (*Choloepus hoffmanni*)

Sara E. Childs-Sanford, DVM, MS, Dipl ACZM,<sup>1\*</sup> Lisa M. Scanlon, MA,<sup>1</sup> Jennifer Higgins, DVM, PhD,<sup>1</sup> Isabel Jimenez, BS,<sup>2</sup> and Andrew J. Makowski, BA<sup>3</sup>

<sup>1</sup>Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA; <sup>2</sup>College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA; <sup>3</sup>Heartland Assays LLC, Ames, IA 50010 USA

### Abstract

Two-toed sloths are unique arboreal members of the superorder Xenarthra, in which disease related to calcium metabolism has been reported.<sup>1-3</sup> This research surveyed multiple biomarkers of calcium and vitamin D metabolism, measured epidermal 7-dehydrocholesterol (7-DHC) concentrations in two locations, and evaluated the use of dried blood spots for the measurement of 25-hydroxyvitamin D (25[OH]D), in Hoffmann's two-toed sloths (*Choloepus hoffmanni*). Samples were collected from nine indoor-housed healthy sloths during routine examinations. Serum was analyzed for 25(OH)D<sub>2</sub>/D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>2</sub>/D<sub>3</sub>, and 24,25(OH)<sub>2</sub>D<sub>2</sub>/D<sub>3</sub> parathyroid hormone (PTH), ionized calcium (iCa), and minerals (calcium [Ca], magnesium [Mg], and phosphorus [P]). Full thickness lumbar and abdominal skin biopsies were analyzed for 7-DHC. Whole blood was collected on a dried blood spot (DBS) card and analyzed for 25(OH)D<sub>2</sub>/D<sub>3</sub>.

All detectable vitamin D metabolites were analogues of D<sub>3</sub>, with mean ± SD of 25.1 ± 7.2 ng/ml for 25(OH)D<sub>3</sub>, 54.2 ± 18.9 pg/ml for 1,25(OH)<sub>2</sub>D<sub>3</sub>, and 7.7 ± 3.2 ng/ml for 24,25(OH)<sub>2</sub>D<sub>3</sub>. Mineral values were within expected ranges. Average PTH was 0.2 ± 0.17 pmol/L and iCa was 1.1 ± 0.14 mmol/L. There was a significantly higher concentration of epidermal 7-DHC in the abdominal (184.4 ng/g) compared to lumbar (94.87 ng/g) biopsy samples (*P* = 0.038). Statistical analysis using Passing-Bablok regression analysis, Bland-Altman plots, and paired *t*-test found good agreement between DBS and serum samples for measurement of 25(OH)D<sub>3</sub>, without constant or proportional bias. This research generates baseline data regarding vitamin D and calcium metabolism in *Choloepus hoffmanni*, and provides a foundation for future research projects in this species designed to improve husbandry and nutrition recommendations and reduce the incidence of disease conditions related to calcium homeostasis.

**Key words:** 7-dehydrocholesterol, *Choloepus hoffmanni*, dried blood spots, two-toed sloth, vitamin D

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## CLINICAL IMPLICATIONS OF DENTAL MALOCCLUSION IN VICTORIAN KOALAS (*Phascolarctos cinereus*)

Kate B. Bodley, BSc(Vet), BVSc, MVS,<sup>1\*</sup> Lyndall M. Pettett, PhD,<sup>2</sup> Marjorie E. Milne, BVSc, FANZCVS (Radiology),<sup>3</sup> David J. Reese, DVM, Dipl ACVR, MRCVS,<sup>4</sup> and Helen E. McCracken, BSc (Vet), BVSc, MVS<sup>1</sup>

<sup>1</sup>Zoos Victoria, Elliott Ave, Parkville, VIC 3052, Australia; <sup>2</sup>School of Veterinary Science, The University of Queensland, Gatton, QLD 4343, Australia; <sup>3</sup>U-Vet Animal Hospital, The University of Melbourne, Werribee, VIC 3030, Australia; <sup>4</sup>VetCT Consultants in Telemedicine Pty Ltd, Fremantle, Western Australia, 6160

### Abstract

Koalas feed exclusively on a fibrous, abrasive diet of *Eucalyptus* spp. foliage. Selective digesta retention in the cecum allows them to feed on leaf with poor nutritive value.<sup>1</sup> Efficient digestion of this material also relies on specialized dental morphology. Koalas possess high, tightly interlocking molar cusps, with opposing long, curved blades, producing an efficient cutting-shearing action.<sup>2</sup> The influence of age-related dental wear on digestive efficiency of koalas, and therefore their longevity, is well-understood;<sup>3,4</sup> however, dental malocclusion is now recognised as a frequent finding in captive and free-ranging koalas in Queensland<sup>5</sup> and South Australia (Pettett, unpublished data).

Since 2007, dental disease has been recognised as a significant cause of morbidity and mortality in the Melbourne Zoo koala collection. Dental malocclusion is now understood to be the primary cause of abnormal dental wear in these cases. Most frequently, upper incisor wear results in pulp exposure and tooth root abscess.

When dental abnormalities are detected, CT scanning is used to identify any osteolytic lesions. Dental extraction plus local antimicrobial therapy (prolonged-release antibiotic copolymer or antibiotic-impregnated polymethylmethacrylate beads) has been the chosen therapeutic approach. However, the prognosis for recovery remains poor. Complications that have developed during treatment include nasolacrimal duct dysfunction, progressive osteomyelitis, severe facial cellulitis, aspiration pneumonia, and gastrointestinal disease. In 2018, Melbourne Zoo veterinarians conducted a population health assessment in free-ranging koalas at Framlingham Forest, VIC, Australia, and identified dental malocclusion in 34 of 162 koalas examined.

**Key words:** Dental malocclusion, koala, osteomyelitis, *Phascolarctos cinereus*

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**A CASE SERIES OF FATAL DISSEMINATED FUNGAL INFECTION IN CAPTIVE-MANAGED BONGO ANTELOPE (*Tragelaphus eurycerus*)**

**Michael M. Garner, DVM, DACVP,<sup>1\*</sup> Daniel V. Fredholm, MS, DVM, Dipl ACZM,<sup>2</sup> Scott B. Citino, DVM, Dipl ACZM,<sup>2</sup> James F. X. Wellehan, Jr., DVM, PhD, Dipl ACZM, Dipl ACVM (Virology, Bacteriology, Mycology), DipECZM (Herpetology),<sup>3</sup> M. Kelly Keating, DVM, DACVP,<sup>4</sup> Jana M. Ritter, DVM, DACVP,<sup>4</sup> Shawn Lockart, PhD, D(ABMM), F(AAM),<sup>4</sup> Colleen Lysen,<sup>4</sup> and Daniel S. Bradway, BS<sup>5</sup>**

<sup>1</sup>Northwest ZooPath, Monroe, WA 98272 USA; <sup>2</sup>White Oak Conservation, Yulee, FL 32097 USA; <sup>3</sup>Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32608 USA; <sup>4</sup>Centers for Disease Control and Prevention, Atlanta, GA 30329 USA; <sup>5</sup>Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA 99164 USA

**Abstract**

Over the span of 6 yr, six adult bongo antelope (*Tragelaphus eurycerus*) from a single institution died without premonitory signs or with brief morbidity and with no historically abnormal medical history. All animals were of the same genetic lineage and in good body condition at the time of death. Gross necropsy findings in all cases included multifocal tan nodules up to 10 cm in diameter that were most numerous in the heart, lung and kidneys. Histologic examination identified these nodules as foci of granulomatous inflammation containing branching, septate, broad, undulating fungal elements. Identification of the fungal species was pursued using PCR with sequencing, immunohistochemistry, and culture. Multiple fungal agents were identified using the various modalities, and commonality of species identification was limited to *Cladosporium* sp. in two of the cases. The overall clinical and pathologic findings in these cases were identical, and considered the same infectious disease process. The *Cladosporium* sp. was considered a candidate as an emerging fatal infectious agent in this population of bongo antelopes. In all animals death was attributed to conduction abnormalities associated with the cardiac lesions.

**Key words:** Bongo antelope, mycosis, pathology, *Tragelaphus eurycerus*

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## PRESUMPTIVE CONGENITAL HYPOTHYROIDISM IN RED PANDAS (*Ailurus fulgens*): A CASE SERIES OF FULL SIBLINGS FROM THREE SUCCESSIVE LITTERS

*Kimberly A. Thompson, DVM, MPVM, Dipl ACVPM, Dipl ACZM,<sup>1\*</sup> Ronan Eustace, DVM,<sup>2</sup> John C. Fyfe, DVM, PhD,<sup>3</sup> and Dalen Agnew, DVM, PhD, DACVP<sup>4</sup>*

<sup>1</sup>*Binder Park Zoo, Battle Creek, MI 49014 USA;* <sup>2</sup>*Potter Park Zoo, Lansing, MI 48912 USA;* <sup>3</sup>*Michigan State University Laboratory of Comparative Medical Genetics, East Lansing, MI 48824 USA;* <sup>4</sup>*Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI 48910 USA*

### Abstract

High neonatal mortality among red pandas (*Ailurus fulgens*) challenge the long-term sustainability of the SSP population.<sup>2,3,5</sup> The majority of neonatal deaths are due to pneumonia and trauma; however, cause of death is unknown in 25% of cases.<sup>2</sup> Congenital hypothyroidism is a rare condition in domestic animals, typically due to an inherited genetic defect.<sup>1,4</sup> Non-goiterous congenital hypothyroidism was presumptively diagnosed in red panda neonates from three successive litters, with identical parentage. The index case was a 5-mo-old cub with hypoplastic thyroids and cretin dwarf appearance on necropsy. The following two litters were each composed of a pair of cubs. A cub died in litter one at 3 wk, and litter two at 3 days; both had hypoplastic thyroids, although cause of death was unrelated. The two surviving cubs (one from each litter) were diagnosed with suspected hypothyroidism at 12 and 9 wk due to failure to grow, elevated TSH, and low total T4, free T4, and total T3. The cubs were treated with levothyroxine 0.022 mg/kg p.o., b.i.d. until 6 mo of age and then changed to s.i.d. The more severely affected cub was hand-reared and had pronounced delays in development: ataxia, bladder atony, gastro-intestinal ileus, and cretin appearance. Both cases had excellent response to treatment and matured to normal adult size and appearance. These cases demonstrate the importance of collecting thyroid tissue, (or proximal trachea/larynx if gross visualization not possible), in neonates for histopathology. Further investigation into the role of thyroid disease in neonatal red panda mortality is warranted.

**Key words:** *Ailurus fulgens*, congenital hypothyroidism, neonate, red panda

### ACKNOWLEDGMENTS

The authors thank the staff at Binder Park Zoo for their dedication in caring for these animals.

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## REPEATED INTRAVENOUS MESENCHYMAL STEM CELL ADMINISTRATION IN TWO CHEETAHS (*Acinonyx jubatus*) WITH VENO-OCCLUSIVE DISEASE

*Matthew E. Kinney, DVM, Dipl ACZM,<sup>1\*</sup> Meredith M. Clancy, DVM, MPH, Dipl ACZM,<sup>1</sup> Lauren L. Howard, DVM, Dipl ACZM,<sup>1</sup> Robert Harman, DVM, MPVM,<sup>2</sup> and Ilse H. Stalis, DVM, DACVP<sup>3</sup>*

<sup>1</sup>*San Diego Zoo Safari Park, Department of Veterinary Services, Escondido, CA 92027 USA;*  
<sup>2</sup>*VetStem, Poway, CA 92064 USA;* <sup>3</sup>*San Diego Zoo Global, Institute for Conservation Research, Disease Investigations, San Diego, CA 92101-0551 USA*

### Abstract

Two zoo-housed cheetahs (*Acinonyx jubatus*) (cheetah A and B) with hepatic veno-occlusive disease diagnosed via laparoscopic liver biopsy were administered allogenic adipose-derived mesenchymal stem cells (MSC) intravenously. MSC were cultured after laparoscopic collection of adipose tissue from the falciform ligament of a donor cheetah with no evidence of hepatic disease. After isolation and culture expansion at a commercial laboratory, mesenchymal stem cells were administered intravenously through an 18-micron filter over 10 min. Both cheetahs received three treatments approximately 3 wk apart with MSC doses increasing from 6 million cells to 18 million cells. A fourth treatment of 36 million cells was administered to cheetah A following severe elevations in hepatocellular leakage enzymes. A fourth treatment of 18 million cells was administered to cheetah B at repeat laparoscopic liver biopsy 6 mo following the first MSC treatment. Physiologic parameters were monitored during MSC administration and no adverse effects were observed during any of the 8 MSC administrations. Cheetah A was euthanized 9 mo following initial laparoscopic liver biopsy, 8 mo following the first dose of MSC, and 16 days following the final dose of 36 million cells. Cheetah B is currently alive 18 mo following initial laparoscopic liver biopsy, 16 mo following the first MSC dose, 8 mo following the most recent dose. Hepatocellular enzymes and serum bile acids remain mild to moderately elevated and are monitored every 1-3 mo.

**Key words:** *Acinonyx jubatus*, cheetah, liver fibrosis, mesenchymal stem cell, veno-occlusive disease

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## USE OF A CONTINUOUS GLUCOSE MONITOR TO AID IN MANAGEMENT OF INSULIN-DEPENDENT DIABETES MELLITUS IN A KOALA (*Phascolarctos cinereus*)

*Cora L. Singleton, DVM, Dipl ACZM,<sup>1\*</sup> Cynthia K. Stadler, DVM,<sup>2</sup> Athena Philis-Tsimikas, MD,<sup>3</sup> Heidi Sharipov, MS, MEng,<sup>4</sup> and Patricia M. Gaffney, DVM, MPVM, PhD, DACVP<sup>5</sup>*

<sup>1</sup>*San Diego Zoo, San Diego Zoo Global, San Diego, CA 92101 USA;* <sup>2</sup>*Los Angeles Zoo, Los Angeles, CA 90027 USA;* <sup>3</sup>*Scripps Whittier Diabetes Institute, San Diego, CA 92121 USA;* <sup>4</sup>*Dexcom, Inc., San Diego, CA 92121 USA;* <sup>5</sup>*Disease Investigations, Institute for Conservation Research, San Diego Zoo Global, San Diego, CA 92112 USA*

### Abstract

A 2.75-yr-old male koala (*Phascolarctos cinereus*) presented with weight loss and polydipsia. Insulin-dependent diabetes mellitus (IDDM) was diagnosed based on serum glucose and insulin values and the homeostasis model assessment (HOMA). Repeated examinations, advanced imaging, hematology, serum biochemistry, and bone marrow cytology failed to detect a cause for IDDM but did reveal anemia with early myelodysplasia.

Treatment with three types of insulin failed to achieve predictable euglycemia, with very low doses occasionally causing hypoglycemia. To understand glucose response to insulin dosing, a continuous glucose monitor (CGM; Dexcom G6®) was placed in the paralumbar region. Interstitial glucose measured by the CGM correlated well with blood glucose values; however, the lifespan of each CGM decreased and episodes of data dropout increased over time.

Approximately 9.5 mo after diagnosis of IDDM, the koala developed pneumonia with rhinitis and otitis. Despite comprehensive treatment, including percutaneous endoscopic gastrostomy tube placement and voluntary nebulization, the koala's health continued to decline. Humane euthanasia was performed.

Post-mortem examination and histopathology confirmed the clinical diagnoses of IDDM, bacterial pneumonia with right middle lung lobe consolidation, and suppurative rhinitis and otitis media. Insulin and glucagon immunohistochemistry confirmed widespread atrophy of pancreatic islets, with severe loss of insulin-secreting beta cells and to a lesser extent alpha cells. Fasciitis and myositis were present at the sites of CGM placement associated with foreign material.

Diabetes mellitus in koalas is rare, with no reports of prolonged therapy. The CGM allowed for periods of improved glycemic control but long-term stable euglycemia was never achieved.

**Key words:** Continuous glucose monitor, diabetes mellitus, koala, pancreatic islet atrophy, *Phascolarctos cinereus*

### ACKNOWLEDGMENTS

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# DERIVATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE, BLOOD, AND PLACENTA AND ADMINISTRATION IN RETICULATED GIRAFFES (*Giraffa camelopardalis reticulata*) FOR TREATMENT OF SEVERE ORTHOPEDIC DISEASE

Valerie Johnson, DVM, DACVECC, MS,<sup>1\*</sup> June Olds, DVM,<sup>2</sup> Liza Dadone, VMD,<sup>3</sup> Matthew Johnston, VMD, DABVP (Avian Practice),<sup>1</sup> and Steven Dow, DVM, DACVIM (Small Animal Internal Medicine), PhD<sup>1</sup>

<sup>1</sup>Colorado State University, Ft. Collins CO 80524 USA; <sup>2</sup>Blank Park Zoo, Des Moines IA 50315 USA; <sup>3</sup>Cheyenne Mountain Zoo, Colorado Springs CO 80906 USA

## Abstract

Orthopedic dysfunction is a common cause of lameness in giraffes in managed care and is a significant cause of morbidity and mortality.<sup>1-3</sup> Multiple etiologies have been proposed to account for this pathologic process but a definitive cause has not been identified.<sup>2,3</sup> This process can be incredibly difficult to manage and involve intricate and risky anesthetic events.<sup>3</sup> While prevention would be preferable to treatment, the multifactorial etiology is still incompletely understood and may not be able to be corrected in a managed care setting. Safe and effective treatments are urgently needed that will provide both symptomatic relief and ideally assist in resolution of the pathologic process. Mesenchymal stem cells (MSC) are a recent novel therapeutic in the field of regenerative medicine that possess the unique qualities of providing symptomatic relief through the downregulation of inflammation, but in addition provide regeneration of the damaged joint through provision of growth factors and stimulation of endogenous stem cell populations.<sup>4</sup> MSC can be derived from a variety of tissues most commonly adipose, bone marrow or placenta. This report describes the generation of mesenchymal stem cells from multiple tissue sources in the giraffe (*Giraffa camelopardalis reticulata*) and subsequent characterization and administration to multiple giraffes with severe orthopedic dysfunction. All giraffes treated were reported to have increased comfort and function as assessed by evaluation of subjective and objective measures. This therapy is a promising new technology that has great potential in the treatment of a challenging and common disorder in this species.

**Key words:** *Giraffa camelopardalis reticulata*, giraffe, osteoarthritis, regenerative medicine, stem cell

## ACKNOWLEDGMENTS

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## INTEGRATIVE MEDICINE FOR GERIATRIC ZOO ANIMALS

*Narda G. Robinson, DO, DVM, MS, FAAMA*

*CuraCore Integrative Medicine & Education Center, Fort Collins, CO 80524 USA*

### **Abstract**

Animals in zoos are living longer, meaning that their needs for physiologic support as well as effective, safe pain management are growing as well. Ensuring that elderly animals experience a positive quality of life can pose challenges, however. Modern animal welfare science asserts that each individual should have some capacity to act independently, to make choices, to solve problems, and to receive rewards.<sup>1</sup> Unfortunately, age-related declines may limit animals' abilities to achieve positive welfare status as a result of pain, mobility impairment, generalized discomfort (visceral or physical), and/or cognitive deterioration.

Toward this end, zoos across the globe are incorporating integrative strategies such as acupuncture, photomedicine (e.g., laser therapy), herbs, and/or massage.<sup>2,3</sup> A multimodal approach that combines one or more of these treatments typically provides the most robust results, delivering benefits through myriad mechanisms.

For example, instead of addressing musculoskeletal pain with potentially harmful medications whose pharmacokinetics and pharmacodynamics can vary widely across species,<sup>4</sup> one could address musculoskeletal pain more effectively and comprehensively through non-pharmacologic means.

That is, acupuncture, photomedicine, and massage each relax muscles, reduce anxiety, and improve circulation through their effects on muscles, fascia, nerves, and vessels. One way in which they work is through non-invasive neuromodulation. This settles neuropathic nerves and counters spinal cord wind-up, which cumulatively correct maladaptive neuroplastic changes resulting from chronic pain states. Connective tissue stretch and mechanoreceptor input from acupuncture and massage promote endogenous analgesia and may invoke long-lasting structural improvements as well.

Another problem of aging zoo inhabitants involves declines in overall body mass. This may arise from pain-related inappetence, alterations in digestive motility and secretions, and/or cognitive and sensory impairment. Acupuncture and certain botanical agents such as ginger assist in the recovery of appropriately orchestrated motility signaling in the digestive tract, which may in itself improve appetite. Photomedicine, acupuncture, and some forms of cannabis improve cognitive function. Certain essential oils employed as aromatherapy may also stimulate mental processes along with appetite.

These are merely a few examples of the many ways in which integrative medicine, based on rational methodologies and science-based mechanisms, promote and maintain positive welfare and health for geriatric zoo animals. Taking a highly individualized, slow, and non-aversive approach

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to each patient is essential, as geriatric animals may require ongoing and regular treatment. Done well, integrative medical care can and should be comfortable, relaxing, and sought by the patient.

**Key words:** Botanical medicine, integrative rehabilitation, massage, photomedicine, veterinary acupuncture

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## **BREATHE EASY: UNDERSTANDING AND HANDLING RESPIRATORY COMPLICATIONS UNDER ANESTHESIA**

*Sathya K. Chinnadurai, DVM, MS, Dipl ACZM, Dipl ACVAA, Dipl ACAW*

*Chicago Zoological Society, 3300 Golf Road, Brookfield, IL 60546 USA*

### **Abstract**

This lecture serves as a refresher on 1) respiratory physiology for non-domestic species 2) monitoring of respiratory function under anesthesia, and 3) common respiratory anesthetic complications with an emphasis on pathophysiology and treatment. We discuss a systematic approach to the diagnosis and treatment of common peri-anesthetic respiratory derangements, such as hypoxemia and hypoventilation.

**Key words:** Anesthesia, respiratory complications

### **INTRODUCTION AND PHYSIOLOGY REVIEW**

We first briefly review the typical mammalian respiratory systems and then discuss the variations on that theme, which are seen in diving mammals, birds, reptiles, amphibians, and fish. The most simplistic and yet most critical aspect of respiratory physiology is knowing that the respiratory system is always performing two primary tasks: 1) absorbing oxygen and 2) eliminating carbon dioxide. The efficiency of the respiratory system at performing those two tasks is directly related to the ratio of respiratory surface area to volume (SA:V) that is available for gas exchange. Variations in SA:V ratio can occur due to taxonomic differences and anesthetic complications, such as atelectasis and pulmonary shunting.

### **ANESTHETIC MONITOR REVIEW**

The primary monitors for assessing respiratory function are the pulse oximeter, capnograph, and blood gas analyzer. Each of those monitors is discussed in more detail below.

**The pulse oximeter:** The pulse oximeter uses photoplethysmography to determine arterial hemoglobin saturation. It uses light emitting diodes and photosensors to detect pulsatile blood flow and determine a ratio of oxygenated to deoxygenated hemoglobin based on differential light absorption. Accurate performance depends on multiple factors including, good tissue perfusion, good pulse quality, thin epidermis, hemoglobin and oxyhemoglobin that absorb light at the required frequencies.<sup>1</sup> Species difference in hemoglobin structure could affect the accuracy of the reading. In cases of anemia, a patient may have excellent hemoglobin saturation, but an overall deficiency in hemoglobin will still result in poor oxygen delivery. Other sources of error include, patient movement, bright ambient light and the fact that most devices are not calibrated to read accurately below 80%.

**The capnograph:** Capnography is arguably the most useful anesthetic monitor and often the most underused. Effective capnography can identify conditions which might lead to hypoxia, before a

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patient is hypoxic. It is one of the most reliable ways of detecting cardiac arrest and airway obstruction. The capnograph can also be used for confirming endotracheal tube placement in challenging intubations, detecting disconnections and leaks and problems with sodalime and one-way valves.

The capnograph typically uses infrared spectrometry to determine partial pressure of CO<sub>2</sub> in expired gas.<sup>1</sup> This generates three pieces of information: 1) end-tidal CO<sub>2</sub> partial pressure, 2) a capnograph waveform, and 3) a respiratory rate. In order for CO<sub>2</sub> to be detected and read out on a capnograph, three steps have to take place:

1. cellular metabolism has to produce CO<sub>2</sub>,
2. circulation has to bring CO<sub>2</sub> from the periphery to the lungs, and
3. the lungs have to be ventilating for the CO<sub>2</sub> to be exhaled and detected by the monitor.

Abnormalities in end-tidal CO<sub>2</sub> can occur due to derangements in any of those three parameters. In a healthy normal animal, end-tidal CO<sub>2</sub> provides a close approximation of arterial partial pressure of CO<sub>2</sub>, the indicator of ventilatory function and respiratory pH balance. Hypoventilation can cause increases in ETCO<sub>2</sub> and PaCO<sub>2</sub>. Hyperventilation can cause decreases in both. There are also numerous scenarios when the discrepancy between expired and arterial CO<sub>2</sub> may grow. These include any state of poor perfusion, which decreases cardiac output and pulmonary perfusion. With less blood delivered to the lungs, there is less CO<sub>2</sub> exhaled, but there is no change in CO<sub>2</sub> production.

Many practitioners use their capnograph simply for one value: end-tidal CO<sub>2</sub>. That single number can be used as an effective indicator of ventilatory function, but the capnograph waveform provides many more valuable pieces of information about equipment and animal function. It can track changes in not only ventilation, but cardiac output and metabolic rate. The four phases of the capnogram that should be interpreted are pictured in Figure 1.

## **BLOOD GAS INTERPRETATION**

What can a blood gas tell you that you did not already know? Both pulse oximetry and capnography provide useful, non-invasive second to second, assessments of ventilation and oxygenation. But both methodologies can have some drawbacks that compromise their accuracy. Direct blood gas analysis can identify abnormalities in species for which non-invasive methodology is not validated. A capnograph can provide a non-invasive assessment of expired CO<sub>2</sub>, which should be similar to arterial CO<sub>2</sub>. Without blood gas analysis, it is possible that an increase in physiologic dead space (causes listed below) could compromise the accuracy of the capnograph. In species capable of pulmonary shunting, such as reptiles, the expired CO<sub>2</sub> may not represent the arterial CO<sub>2</sub> at all.

Basic interpretation should be a straightforward, step-by-step process taking 30-60 seconds. While there are more advanced ways of interpreting acid-base changes in human and veterinary critical care, they are not covered here.

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Six-step interpretation:

- 1) What is the pH? Normal pH for most mammals is 7.35-7.45. Is the patient's pH low (acidemia) or high (alkeleemia)?
- 2) What is the PO<sub>2</sub> (arterial sample)? Normal arterial PO<sub>2</sub> 100 mmHg, while breathing room air. Is the patient hypoxemic? Is the patient's oxygen tension appropriate for the fraction of oxygen that it is breathing?
- 3) What is the PCO<sub>2</sub>? Normal PCO<sub>2</sub> is 35-45 mmHg. PCO<sub>2</sub> represents the respiratory component of the acid base derangement. Changes in PCO<sub>2</sub> result in respiratory acidosis and alkalosis.
- 4) What is the metabolic component (HCO<sub>3</sub> and base excess)? Are the changes appropriate for the changes in PCO<sub>2</sub> or is there a metabolic acidosis or alkalosis?
- 5) What is the level of compensation? Derangements of either respiratory or metabolic acid-base balance often result in compensatory change from the other system (i.e., metabolic acidosis often results in a compensatory respiratory alkalosis [hyperventilation]).
- 6) Are there abnormalities in electrolytes or lactate?

An in-depth discussion of each of the six steps above is provided in the following text.

1) pH: pH only gives us the direction and extent of the derangement, but does not tell us the source of the problem. It does help narrow down the differentials for the primary problem and the list of actions that need to be done to correct the problem. Carnivores tend to have slightly more acidic pH, while herbivores and omnivores with high carbohydrate diets tend to have more alkaline blood pH.

2) PaO<sub>2</sub>: Normal PaO<sub>2</sub> (arterial partial pressure of oxygen) is 100 mmHg when breathing room air and 400-500mmHg when breathing 100% oxygen. Hypoxemia is defined as a PaO<sub>2</sub> < 80 mmHg. Calculating an alveolar-to-arterial oxygen gradient (A-a gradient) provides useful information about the cause of the hypoxemia. In addition, calculating a PaO<sub>2</sub>/FiO<sub>2</sub> (partial pressure to fraction of inspired oxygen) ratio provides a very easy means of assessing pulmonary function. The A-a gradient is most accurate when the patient is breathing room air (21% oxygen) while the PaO<sub>2</sub>/FiO<sub>2</sub> ratio can be done with any FiO<sub>2</sub>.<sup>3</sup>

A measured hypoxemia is typically the result of one of five problems:

- i. Hypoventilation: the patient is not breathing frequently or deeply enough. In cases of hypoventilation, the PaO<sub>2</sub> is low but there is a normal A-a gradient.
- ii. Low FiO<sub>2</sub>: The inspired percentage of oxygen is too low. This is rare as most anesthetized animals are breathing an enriched oxygen mixture. The animal is hypoxemic with a normal P/F ratio
- iii. Ventilation/perfusion mismatching: This is common and is likely the main source of hypoxemia in anesthetized large animals. It is often associated with atelectasis and can be exacerbated by poor cardiac output and poor pulmonary perfusion.
- iv. Diffusion impairment: Rare, is not discussed here.
- v. Anatomic right to left shunt: Rare, is not discussed here.

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3)  $PCO_2$ : Carbon dioxide tension quantifies the balance between cellular metabolism and alveolar ventilation. Hypercapnea typically results from a decrease in ventilation, but can be a result of increased metabolism (exertion). Hypocapnea could be from hyperventilation or decreased metabolic activity.  $PCO_2$  can also be compared to end-tidal  $CO_2$  to determine if there is an increase in physiologic dead space. End-tidal  $CO_2$  should slightly underestimate arterial  $CO_2$  by 5 mmHg. An increase in this difference indicates that there are areas of lung that are ventilated, but not perfused. This occurs with pulmonary thromboembolism and decreased pulmonary perfusion secondary to decreased cardiac output.

4) Bicarbonate and base excess: Both of these calculated parameters provide information about metabolic alkalosis or acidosis. These are indirect measures as both are derived from the measured  $CO_2$  on the blood gas (formulas given above). A typical reference range for  $HCO_3$  is 19-24 mEq/L. To account for the effect of  $CO_2$  on  $HCO_3$  calculation, base excess (BE) can be used. Base excess determines the amount of bicarbonate that needs to be added to blood to bring the pH back to 7.4, when  $PCO_2$  is set at 40. Essentially, base excess factors in the effect of body buffer systems and factors out the effect of  $CO_2$  on bicarbonate to determine the metabolic contribution to acid-base balance. Reference interval for bicarbonate is -4 to 4 mEq/L. While not a perfect system, BE provides a rapid way of determining the metabolic disturbance. If BE is high, there is a metabolic alkalosis and if it is low there is a metabolic acidosis, regardless of the respiratory disturbance.

5) Compensation: Before evaluating compensation, look back at the pH. If the pH is low, the primary process is an acidosis and the compensatory process (if present) is an alkalosis. Compensation rarely brings the patient back to a normal pH and never overcompensates. A primary chronic respiratory acidosis (hypoventilation) will lead to a compensatory metabolic alkalosis, but pH will not return to normal and will not become alkalotic. Methods of compensation include chemical buffers (few seconds), respiratory (few minutes) and metabolic compensation (few days).<sup>3</sup>

6) Lactate and electrolytes: Blood lactate is a by-product and indicator of anaerobic metabolism. Increases in blood lactate typically accompany decreases in tissue perfusion. This could include ischemic muscle from a positional or exertional myopathy in which metabolic oxygen demand has outstripped the available oxygen delivery. Focal ischemia (strangulated intestine, compromised blood flow to a limb after trauma) can also increase lactate production, and in some cases the hyperlactatemia will only be seen after perfusion is reestablished. Electrolyte interpretation is similar to routine chemistry interpretation.

This step-by-step process should lead to an assessment of oxygenation and acid-base disturbance. Acid-base disturbances are described by the pH change (acidosis or alkalosis) and the source of the disturbance (metabolic or respiratory). In many cases, there is a mixed metabolic and respiratory disturbance. A few causes of the four main acid-base disturbances in animals are listed:

*Metabolic acidosis:* Gastrointestinal bicarbonate loss (diarrhea), renal bicarbonate loss, lactic acidosis secondary to hypoperfusion.

*Metabolic alkalosis:* Pyloric outflow obstruction, excessive exogenous bicarbonate therapy.

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*Respiratory acidosis:* Hypoventilation due to anesthesia, muscle relaxation, central nervous system (especially medullary or cervical) disease, airway obstruction, excessive dead space ventilation or hyperthermia.

*Respiratory alkalosis:* Hyperventilation due to hypoxemia, pain, anxiety, inappropriate ventilator settings.

## RESPIRATORY COMPLICATIONS UNDER ANESTHESIA

Hypoxia: In terrestrial mammals, hypoxemia is defined as an arterial partial pressure of oxygen less than 60 mmHg or and SpO<sub>2</sub> of less than 90%. Hypoxia occurs for one of five reasons:

1. True hypoventilation: Either the patient is not breathing enough or it has an obstruction, keeping it from breathing. In these cases, the patient has a high PaCO<sub>2</sub> and a low PaO<sub>2</sub>
2. Ventilation/perfusion mismatch:
  - a. Atelectasis
  - b. Dead space ventilation
3. Anatomic right-left shunt: uncommon, unless you are a reptile and are built for this
4. Low inspired FiO<sub>2</sub> (the animal is breathing a low oxygen mixture): this is rare under anesthesia
5. Diffusion impairment: Very rare

Of the above choices, 1 and 2 are, by far, the most common. Hypoventilation can be ruled in or out only with a capnograph or blood gas to determine CO<sub>2</sub>. If the ETCO<sub>2</sub> is high and the SpO<sub>2</sub> is low, breathe more frequently. If the ETCO<sub>2</sub> is low or normal and the SpO<sub>2</sub> is low, there is most likely a ventilation-perfusion mismatch, such as atelectasis. In this case fewer, deeper, larger breaths will help but only increasing rate will not.

Tachypnea: Again, we often assume that if a patient's respiratory rate increases, that it is light. In truth, there are likely four reasons why a patient is tachypneic under anesthesia:

1. It is hypercapneic
2. It is hypoxemic
3. It is light and responding to noxious stimulus
4. It is hot and trying to lose heat

Either CO<sub>2</sub> is too high, oxygen is too low, the patient is light/stressed/painful (i.e. inadequately anesthetized) or it is too hot. Instinctually turning up the gas only addresses one of these options. When noticing any change in respiratory rate and character, do a spot check. What is the ETCO<sub>2</sub>, the SpO<sub>2</sub>, body temperature and depth of anesthesia? If something does not make sense, run a blood gas and make sure your equipment is working.

In most recumbent animals under anesthesia, there is a degree of dependent atelectasis and hypostatic vascular congestion, combined with some preferential blood flow to gravitationally dependent areas of lung. This combination of issues is what can lead to profound ventilation/perfusion mismatching under anesthesia, as the dependent areas of lung are

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underinflated or collapsed, but receiving the majority of the bloodflow, making gas exchange very inefficient.

Atelectasis: Under anesthesia, especially with 100% oxygen, there is a significant degree of regional dependent atelectasis in almost any mammal. This is usually regional alveolar collapse, not larger lung or lobar collapse. Oxygen (100%) exacerbates this collapse because oxygen is freely diffusible into the blood, while room air (mostly nitrogen) is not. In an animal with a lung full of 100% oxygen, even a brief apnea will result in passive movement of oxygen out of the alveolar space, into the capillary blood. Once all that oxygen has gone from the alveolus to the blood, the alveolus can collapse, causing absorption atelectasis. Under anesthesia, the only way to reliably prevent this is using positive pressure ventilation with some peak end expiratory pressure (PEEP). In the same animal breathing room air (78% nitrogen, 21% oxygen) during the same apneic period, only the 21% oxygen would absorb into the blood leaving the 78% nitrogen skeleton to hold the alveolus open. This is why, in people (and in horses at some institutions), inhalant anesthetics are given in 40% oxygen/60% medical air, and not given in 100% oxygen.

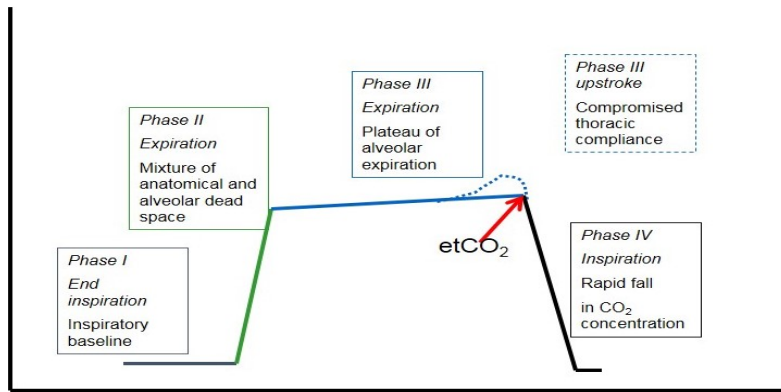
Regarding hypostatic congestion: Under anesthesia, the vasodilation and poor peripheral blood flow that can occur, can result in interstitial (less likely within the alveolus) fluid accumulation and blood pooling. This can actually be a huge problem when an anesthetized large animal is flipped from one lateral recumbency to the other under anesthesia. Depending on the anesthetics used, the animal may not have intact vasomotor tone or sufficient baroreceptor response to redirect blood flow quickly and the change in body position can cause profound drops in cardiac output due to poor venous return to the heart (from the areas of pooling blood). In addition, the atelectatic previously down lung may not have time to adequately reinflate before the other lung starts to become atelectatic, resulting in worsening hypoxia.

## MECHANICAL VENTILATION

In many cases, the most effective way to combat atelectasis and hypoventilation is to use mechanical ventilation. We conclude by briefly outlining the types of mechanical ventilators available and their use.

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**Figure 1.** Phases of the capnogram.

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## **RUBBISH IN, RUBBISH OUT: GETTING THE MOST OUT OF PATHOLOGY**

*Mary Duncan, BVMS, PhD, Dipl ACVP, MRCVS*

*Saint Louis Zoo, Saint Louis, MO 63110 USA*

### **Abstract**

You submitted a biopsy or cytology sample and receive back a report stating “non-diagnostic sample” and it is probable that the pathologist muttered “rubbish in, rubbish out.” My goal is to improve the level of diagnostic reports you receive.

**Key words:** Necropsy, pathology, sample

### **RUBBISH IN**

Take the sample at the border of the lesion with normal tissue (center of the lesion is likely necrotic, organisms are most commonly seen at the interface). Submit a picture of the lesion prior to surgery with the biopsy. Invite the pathologist to view. Images and inks are helpful for sample orientation.

Tiny biopsies taken from the gastrointestinal tract should be placed on a foam pad in cassette, folded in lens paper, to create “teabags.” Separate out left and right, stomach pylorus, and antrum into separate cassettes if precise location is important.

Carcasses should be placed under refrigeration as soon as possible (not frozen). If tissue is severely autolyzed (e.g, reptile under heat lamp, furry mammal died overnight in summer heat), histology may not be worthwhile and possibly an unnecessary expense.

Euthanasia solution will cause discoloration and alteration in consistency of tissues, generally the affected tissue is not of diagnostic quality.

### **WHY DO A NECROPSY ANYWAY?**

Pesky cases may finally make sense. The cause of death may be identified, and other problems might come to light. It provides a review of the anatomy. Necropsies are way more fun than doing budgets. At an Association of Zoos and Aquariums accredited institution, you are required to conduct necropsies.

### **WHAT SAMPLES SHOULD I COLLECT AT NECROPSY?**

Is a specific protocol available for the species? Species Survival Plans may provide information. If you’ve never done a necropsy on a fill-in-the-blank species, where should you begin?

Keeping things together. Use jars (left and right) and bouffant surgery caps.

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## **HOW SHOULD I SAVE MY SAMPLES?**

There are available fixatives that vary in benefits, pros and cons, length of storage, and safety of use. Ideally for good fixation, a sample should be only up to 1cm in dimension. Frozen samples can include multiple small pieces.

### **FOLLOW A ROUTINE**

A submission protocol should be followed. Cases for necropsy should be submitted with some background information, including identification (e.g., prosector will check against bands, notches), origin, status, history (e.g., it is helpful to know if the animal was under treatment, was anorexic), weight, radiographs, natural death or euthanized (if euthanized, the method and route).

A log book should be maintained, either hard copy or electronically. Keep a cheat sheet, take pictures (particularly if you have a team involved on a large case), so no one person will get to see everything (e.g., text pictures).

Bank tissues. In collecting samples you are going to collect a routine list to bank, this allows you to build up a collection of normals for comparison to lesions. Likewise if one of a pair of organs looks normal you will still collect tissue for comparison.

### **NECROPSY ON-SITE, AWAY FROM NECROPSY ROOM**

Keep a checklist so you can pull equipment together quickly (e.g., extension cords, barrels).

### **WRITE A REPORT**

More important than the jargon, the detailed descriptions written in the present tense, is that you get some kind of report entered as soon as possible after the necropsy, since the longer you leave it the poorer your recall (cheat sheets will help). This is of particular importance when there has been a team effort on a large case (you may even want to designate a scribe), when different people may take the lead on different body systems. Make a rough copy and have others add their input before a final gross report is circulated.

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## MITIGATING THE EFFECTS OF LIVE TRADE IN ENDANGERED SPECIES: NUTRITIONAL REHABILITATION OF GREY CROWNED CRANES IN RWANDA

Barry K. Hartup, DVM, MS, PhD<sup>1\*</sup> and Olivier Nsengimana, DVM, MVetSci<sup>2</sup>

<sup>1</sup>Department of Conservation Medicine, International Crane Foundation, Baraboo, WI 53913 USA; <sup>2</sup>Rwanda Wildlife Conservation Association, Kigali, Rwanda

### Abstract

The grey crowned crane (*Balearica regulorum*) is the only species of crane in Rwanda and faces serious threat from domestic live trade. We conducted a study of the nutritional challenges of cranes coming from illegal captivity and the short-term effects of a balanced, formulated diet used to condition the cranes prior to release in the wild. Twelve cranes were randomly selected from 18 individuals that met inclusion criteria. Nutritional analyses from blood samples were conducted before and immediately following provision of Mazuri® Crane Diet for 6 wk while in a quarantine holding facility. We analyzed serum chemistries (8 analytes), serum triglycerides and non-esterified fatty acids, plasma vitamin A (retinol) and vitamin E ( $\alpha$ -tocopherol), and 12 whole blood trace element concentrations. Many cranes were hypoproteinemic or observed with deficiencies in vitamin A or E, selenium or zinc at the beginning of the trial. We observed statistically significant increases in serum total protein, uric acid, cholesterol, triglycerides, plasma vitamin A and E, copper, iron, selenium and zinc by the end of the trial (Wilcoxon signed rank  $P < 0.05$ ). Blood lead declined to baseline levels in two cranes with elevated concentrations ( $> 5 \mu\text{g/dl}$ ) at the beginning of the trial. The results suggest that the current rehabilitation and biosecurity management process is effective in reversing nutritional deficiencies from captivity and improving the condition of cranes prior to release. We expect these effects will improve the success of assimilating the cranes to native habitat following their soft release.

**Key words:** *Balearica regulorum*, diet, grey crowned crane, nutrition, Rwanda, vitamin A

### ACKNOWLEDGMENTS

The authors thank all staff and volunteers of the Rwanda Wildlife Conservation Association, Gorilla Doctors, and the Rwandan Development Board Conservation Department for their assistance with the cranes. ICF Veterinary Research Intern K. Rayment assisted greatly with data analysis. This research was funded in part by an AZA Conservation Fund Grant and the Disney Conservation Fund to B. Hartup.

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## COMPARISON OF A POINT-OF-CARE BLOOD URIC ACID METER TO A STANDARD BENCHTOP CHEMISTRY ANALYZER IN HEALTHY AQUARIUM-HOUSED MAGELLANIC PENGUINS (*Spheniscus magellanicus*)

Julie D. Sheldon, DVM, MS,<sup>1,2\*</sup> Matthew O'Connor, DVM,<sup>1</sup> Caryn P. Poll, DVM,<sup>1</sup> and William Van Bonn, DVM<sup>1</sup>

<sup>1</sup>A. Watson Armour III Center for Animal Health and Welfare, John G. Shedd Aquarium, Chicago, IL 60605 USA; <sup>2</sup>Illinois Zoological and Aquatic Animal Residency Program, Urbana, IL 61802 USA

### Abstract

Uric acid is a primary clinicopathologic marker of renal function in birds and reptiles. Point-of-care uric acid meters are available for human gout management, providing rapid results using only a drop of blood.<sup>1,2</sup> Utility of these meters in animals have yet to be investigated. Small patient size of some birds and reptiles can limit blood volume safely and easily collected; thus, this technology would allow rapid assessment of non-mammalian uric acid concentration with minimal blood volume. Whole blood uric acid concentrations were measured immediately after jugular venipuncture in 21 fasted healthy adult Magellanic penguins (*Spheniscus magellanicus*) using the UASure<sup>®</sup> Blood Uric Acid Meter (UASure<sup>®</sup>, Atwood, CA, 92811, USA). Plasma uric acid concentrations were measured that day at IDEXX via bench-top chemistry analyzer (Beckman AU5800, Beckman Coulter, Inc., Brea, CA, 92821, USA). Mean  $\pm$  SD uric acid concentrations for the UASure<sup>®</sup> and IDEXX results were  $6.1 \pm 1.3$  and  $6.9 \pm 2.0$  mg/dl, respectively. Passing-Bablok regression analysis revealed systematic (intercept = -3.2, 95% CI: -6.5 to -0.56) and proportional differences (slope = 1.7, 95% CI: 1.2-2.2) between methods. A Bland Altman plot revealed a mean negative bias of  $-0.81 \pm 1.1$  mg/dl between the methods. As uric acid concentration increased, the difference between UASure<sup>®</sup> and IDEXX results increased and became more negative. All values fell within reference intervals for this species and the differences were deemed not clinically significant on an individual basis.<sup>3</sup> While the two methods were not in statistical agreement for Magellanic penguins, the UASure Blood Uric Acid Meter has potential for rapidly estimating uric acid concentration, especially in emergency cases.

**Key words:** Magellanic penguin, point-of-care, *Spheniscus magellanicus*, uric acid

### ACKNOWLEDGMENTS

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## PROSPECTIVE ASSESSMENT OF AVIAN COAGULATION IN RESPONSE TO PARENTERAL ADEQUAN<sup>®</sup> ADMINISTRATION IN A DOMESTIC CHICKEN (*Gallus gallus*) MODEL

*Amanda M. Wonn, BS,<sup>1\*</sup> Marjory B. Brooks, DVM, DACVIM (Small Animal Internal Medicine),<sup>2</sup> and Kathryn C. Gamble, DVM, MS, Dipl ACZM, DipECZM (Zoo Health Management)<sup>1</sup>*

*<sup>1</sup>Lincoln Park Zoo, Chicago, IL 60614 USA; <sup>2</sup>Comparative Coagulation Section, Animal Health Diagnostic Center, Cornell University, Ithaca, NY 14853 USA*

### Abstract

Adequan<sup>®</sup> (Elanco, Luitpold Animal Health, Greenfield, IN 46410 USA; Canine, 100 mg/ml) is a parenteral polysulfated glycosaminoglycan approved for use in managing osteoarthritis in dogs and horses. Its chemical structure, similar to heparin, may confer anticoagulant properties.<sup>6</sup> While used extra-label for many species, anecdotal reports describe hemorrhagic diathesis and cardiovascular shock in treated birds;<sup>1</sup> however, in one zoological practice this product has been administered to 36 individuals of 24 avian species since 2002 with good clinical benefit for periods of greater than 12 mo duration (Gamble, personal communication). The empiric dose regimen is weekly for 4-6 wk based on patient improvement, then for two doses at 2-wk intervals, and then maintained monthly. It has been administered subcutaneously, rather than intramuscularly, to reduce potential muscle damage and minimize hemorrhage, as described in a 2012 report.<sup>1</sup>

In this prospective study, Adequan<sup>®</sup> (1 mg/kg SC) was administered to six adult domestic chickens (*Gallus gallus*) following this practice's clinical avian regimen. Patient-side whole blood activated clotting time (ACT) and citrated plasma thrombin clotting time (TCT) submitted to a diagnostic laboratory were used to monitor coagulation status.<sup>2,4</sup> Both ACT and TCT are sensitive to heparin's anticoagulant action, as detected by prolongation of fibrin clot endpoint.<sup>3,5,7</sup> Baseline coagulation parameters varied among birds, thus individual changes over time were evaluated to provide evidence of a heparin-like effect of Adequan and assess use of these assays in future studies in exotic avian species. Ultimately, the goal is to prospectively monitor individual patients to minimize risk of clinical coagulopathy during treatment with Adequan<sup>®</sup>.

**Key words:** Activated clotting time (ACT), Adequan<sup>®</sup>, coagulation, domestic chicken, *Gallus gallus*, thrombin clotting time (TCT)

### ACKNOWLEDGMENTS

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## INDUCING MOLT IN THREE SPECIES OF BIRDS USING NUTRITIONAL METHODS

*Timothy Brunner, BS,<sup>1\*</sup> Anne Tieber, BS,<sup>2</sup> Amanda Hausman, BS,<sup>2</sup> Sydney Oliveira, BS,<sup>2</sup> and Debra Schmidt, MS, PhD<sup>2</sup>*

*<sup>1</sup>Purdue College of Veterinary Medicine, West Lafayette, IN 47906 USA; <sup>2</sup>Saint Louis Zoo, Saint Louis, MO 63110 USA*

### Abstract

Forced molting has been well documented in domestic species, like poultry;<sup>1</sup> however, its effectiveness has not been documented in exotic species, outside of penguins.<sup>2</sup> Using an anecdotal method that has had success previously, but was never documented, we attempted to induce molt in three species of birds, 2.0 beautiful fruit doves (*Ptilinopus pulchellus*), 0.1 spectacled owl (*Pulsatrix perspicillata*), and 1.0 golden white-eye (*Cleptornis marchei*). These three species were selected as all had received full medical workups with no obvious signs of illnesses, and yet were still unable to molt and had poor feather quality. For each bird, food intake was quantified for ten days to establish baseline intakes. Diets were weighed, before and after being offered, in grams. A desiccation plate was placed near the enclosure and weighed daily to account for moisture lost to the environment. The amount of diet offered was then reduced to either the average intake amount or the minimal amount of kcals needed to maintain their body weight as measured over the ten day period. The same nutritionally balanced proportions were maintained. This reduced diet was fed for a period of 14 days. Following this reduction period, the diet was increased to the original amount offered and measured for 4 wk, or until molting occurred. By day 19 of the return to normal diet, the golden white-eye was molting wing feathers and growing neck feathers that were originally absent. The owl received two mice during the initial baseline data collection, but when calculating her caloric needs, we realized she needed three mice daily. We used the baseline data as her reduced diet period and began offering her three mice when the others were starting their reduced diet. By day 33, the spectacled owl was noted to have emerging wing feathers. Unfortunately, by the 4-wk mark, neither of the beautiful fruit doves had shown any signs of molting. From the reduced diet stage to the return to normal diet, there was a 32.9% and 20.5% increase in daily intake for the golden white-eye and spectacled owl, respectively. Overall, this study represents a possible way to induce molt in exotic birds when medical intervention is not working.

**Key words:** *Cleptornis marchei*, golden white-eye, induced molting, *Pulsatrix perspicillata*, spectacled owl

### ACKNOWLEDGMENTS

The authors thank the entirety of the staff in the Bird Department at the Saint Louis Zoo for their assistance in the care and feeding of these birds.

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## EVALUATION OF FIBRINOGEN AND ERYTHROCYTE SEDIMENTATION RATE AS INDICATORS OF INFLAMMATION IN THE AFRICAN PENGUIN (*Spheniscus demersus*)

Barbara J. Mangold, DVM,\* Jennifer E. Flower, MS, DVM, Dipl ACZM, Anne L. Gilewski, LVT, and Allison D. Tuttle, DVM, Dipl ACZM

Mystic Aquarium, Mystic, CT 06355 USA

### Abstract

The use of fibrinogen and erythrocyte sedimentation rate (ESR) as diagnostic tools have been studied in both clinically healthy and diseased avian species as both parameters are non-specific markers of inflammation. Increases in fibrinogen can be seen in inflammatory pathologies associated with trauma, neoplasia, and sepsis and ESR has been shown to increase in pigeons infected with *Plasmodium relictum*.<sup>2,3</sup> Correlations between hyperfibrinogenemia and heterophilia in avian species has been documented to be associated with bacterial infections.<sup>1</sup> The goal of this study is to establish baseline levels for ESR and fibrinogen in an aquarium population of African penguins (*Spheniscus demersus*) and determine if either of these parameters correlate with other indicators of inflammation in this species including white blood cell count (WBC), absolute heterophil count, total protein concentration, and packed cell volume. Sixty-two samples from 25 penguins were evaluated. Fibrinogen ranged from 240-714 mg/dl with a mean of 403 mg/dl. ESR ranged from 0-4 mm/hr with a mean of 0.59 mm/hr. Preliminary data show a positive correlation between WBC count and fibrinogen ( $R = 0.42$ ) and absolute heterophil count and fibrinogen ( $R = 0.57$ ). No other positive correlations were identified. Results of this study suggest that fibrinogen may be a useful adjunct diagnostic for measuring inflammation in this species.

**Key words:** African penguin, erythrocyte sedimentation rate, fibrinogen, inflammatory, *Spheniscus demersus*, white blood cell

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## ULTRAVIOLET FLUORESCENCE AS A NON-INVASIVE DIAGNOSTIC TOOL FOR OPHIDIOMYCOSIS IN LAKE ERIE WATERSNAKES (*Nerodia sipedon insularum*)

*Kathryn Vivirito, MPH,<sup>1\*</sup> Ellen Haynes, DVM,<sup>1</sup> Laura Adamovicz, DVM, PhD,<sup>1</sup> Allison Wright, MS,<sup>1</sup> Kristin Stanford, PhD,<sup>2</sup> Emma Scott,<sup>2</sup> and Matthew C. Allender, DVM, MS, Dipl ACZM, PhD<sup>1</sup>*

<sup>1</sup>*Wildlife Epidemiology Lab, University of Illinois at Urbana-Champaign, Urbana, IL 61802 USA;* <sup>2</sup>*Stone Laboratory, Ohio State University, Put-in-Bay, OH 43456 USA*

### Abstract

Diagnosis of ophidiomycosis (*Ophidiomyces ophiodiicola*) currently requires one or a combination of (1) a positive quantitative PCR (qPCR) result from a skin swab, (2) a positive fungal culture, or (3) characteristic histopathology lesions from a skin biopsy. While reliable, these methods can have a long turnaround time. Long-wave ultraviolet (UV) light has been used to diagnose fungal infections, most recently white-nose syndrome in bats. If applicable to ophidiomycosis, UV light could provide a reliable field-applicable diagnostic tool. Fluorescence intensity from UV light (Phillips Burton Model UV502, 120V) was compared to qPCR analysis to detect *O. ophiodiicola* infections in 38 Lake Erie watersnakes (*Nerodia sipedon insularum*). Photos of snake skin were taken under UV exposure and analyzed to obtain a fluorescence intensity (FI) measurement (ImageJ, v1.52a, National Institute of Health, USA). An FI value of 105u determines whether a lesion visually fluoresces with 74% accuracy (specificity = 95%, sensitivity = 68%). Areas of skin that were exposed to UV light were swabbed and analyzed by qPCR to determine fungal quantity. FI of skin lesions under UV light was compared to qPCR results: FI values from 105-147.7u were likely to be qPCR positive for *O. ophiodiicola*, with an accuracy of 77% (sensitivity = 80%, specificity = 77%). This evidence supports the use of visual fluorescence identification as a limited method to diagnose Ophidiomycosis in Lake Erie watersnakes. Ultimately, qPCR of a skin swab should be performed in conjunction with fluorescence for a definitive diagnosis, but UV light can provide a preliminary, non-invasive, and field-applicable method to detect the presence of *O. ophiodiicola*.

**Key words:** Lake Erie watersnake, *Nerodia sipedon insularum*, *Ophidiomyces ophiodiicola*, ophidiomycosis, ultraviolet (UV) fluorescence

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## CHARACTERIZING QUANTITATIVE PCR INHIBITION DUE TO MELANIN AND HUMIC ACID FOR THE DETECTION OF *Ophidiomyces ophiodiicola*

Allison Wright, MCM,<sup>1\*</sup> Ellen Haynes, DVM,<sup>1</sup> and Matthew C. Allender, DVM, MS, PhD, Dipl ACZM<sup>1,2</sup>

<sup>1</sup>Wildlife Epidemiology Lab, University of Illinois College of Veterinary Medicine, Urbana, IL 61802 USA; <sup>2</sup>Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign, Champaign, IL 61820 USA

### Abstract

Ophidiomycosis, first described in 2006, poses a threat to the health of wild snake populations in the United States.<sup>2</sup> Currently, quantitative PCR (qPCR) of cutaneous swabs is the preferred method for detection of the causative fungus, *Ophidiomyces ophiodiicola*;<sup>1</sup> however, swabs may be contaminated with substances that inhibit qPCR. For example, humic acid, found in the environment, and melanin, found within snake skin, have been shown to yield falsely low DNA quantities when present in qPCR reactions.<sup>3,6</sup> The objective of this study was to characterize the inhibition of an *Ophidiomyces*-specific qPCR assay by humic acid and melanin. We ran qPCR reactions with DNA concentrations ranging from 10<sup>1</sup> to 10<sup>7</sup> fungal copies, and melanin or humic acid concentrations ranging from 0.5-50 ng/μl. Melanin concentrations of 4-10 ng/μl completely inhibited qPCR only at the lowest DNA dilution, while concentrations above 10 ng/μl were completely inhibitory across all DNA concentrations. Humic acid concentrations 5-15 ng/μl completely inhibited qPCR at the lowest DNA dilution, while concentrations > 15 ng/μl were inhibitory across all DNA concentrations. Partial inhibition was observed at the lowest *Ophidiomyces* DNA dilution by 1.5 ng/μl of melanin and 2.5 ng/μl of humic acid, and qPCR efficiency declined as inhibitor concentration increased. These results demonstrate that inhibitors in environmental samples, such as melanin and humic acid, can alter interpretation of qPCR results for *O. ophiodiicola*. This inhibition can result in underestimation of the prevalence and distribution of Ophidiomycosis, leading to significant management consequences.<sup>4,5</sup>

**Key words:** Humic acid, melanin, *Ophidiomyces ophiodiicola*, ophidiomycosis, qPCR inhibition, snake fungal disease

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## MEASURING BLOOD LACTATE CONCENTRATIONS FOLLOWING CAPTURE BY A CANINE SEARCH TEAM IN EASTERN BOX TURTLES (*Terrapene carolina carolina*)

**Katy Klein, BS,\* Laura Adamovicz, DVM, PhD, and Matthew C. Allender, DVM, MS, PhD, Dipl ACZM**

**Wildlife Epidemiology Laboratory, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 USA**

### Abstract

Studies to assess wildlife health commonly evaluate clinical pathology changes, immune responses, pathogen presence, and contaminant exposure, but novel modalities are needed to characterize the unique physiologic responses of reptiles.<sup>1-3,5,10,11</sup> Lactate is an indicator of hypoperfusion and/or anaerobic respiration and can be quickly measured using a point-of-care analyzer.<sup>4,6-9</sup> This study evaluated baseline blood lactate concentrations in free-living eastern box turtles (*Terrapene carolina carolina*,  $n = 105$ ) using a point-of-care analyzer (Lactate Plus Meter, Nova Biomedical Corporation, Waltham, MA, 02454, USA), then determined the effect of handling time, physical examination (PE) abnormalities, and qPCR pathogen detection (*Terrapene* herpesvirus 1, *Mycoplasma* sp., *Terrapene* adenovirus) on lactate levels. Baseline blood lactate concentrations were higher in the spring than summer, in turtles with *Terrapene* herpesvirus 1 ( $n = 11$ ), and in turtles with aural abscesses ( $n = 7$ ) ( $P < 0.05$ ). Lactate concentrations increased between initial capture and PE, with peak values reached 129 min following capture. Lactate at PE was positively associated with baseline lactate levels and packed cell volume and was higher in turtles that remained in their shells ( $P < 0.05$ ). Turtles with pathogens or PE abnormalities may have alterations in blood flow, oxygen delivery, or activity levels, driving increases in baseline lactate.<sup>4,6</sup> Increased handling time likely leads to more escape behaviors and/or breath holding, causing turtles to undergo anaerobic metabolism and raising lactate concentrations.<sup>4,8,9</sup> Overall, lactate measured by a point-of-care analyzer shows variability due to capture and health factors in eastern box turtles and may be a useful adjunctive diagnostic test in this species.

**Key words:** Anaerobic metabolism, blood lactate, eastern box turtle, point-of-care analyzer, reptile, *Terrapene carolina carolina*

### ACKNOWLEDGMENTS

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## RADIOGRAPHIC EVALUATION OF A SPINAL OSTEOARTHROPATHY EPIZOOTIC IN SNAKES SPECIES AT THE SAN DIEGO ZOO

*Elizabeth Bicknese, DVM, MPVM, cVMA,<sup>1\*</sup> Steven Kubiski, DVM, PhD, DACVP,<sup>2</sup> and Meg Sutherland-Smith, DVM, Dipl ACVM<sup>1</sup>*

<sup>1</sup>*Veterinary Services, San Diego Zoo, San Diego, CA 92112-0551 USA; <sup>2</sup>San Diego Zoo Global, San Diego, CA 92112-0551 USA*

### Abstract

Since 2014, over 90 snakes at the San Diego Zoo have necropsy diagnoses of spinal osteoarthropathy based on histologic and radiographic evaluations. While infectious agents and associated inflammation were seen occasionally, the majority of cases were non-inflammatory, making non-infectious etiologies a consideration. A prospective clinical and radiographic evaluation of snakes on over 250 snakes was initiated. Serial assessments in a large subset of snakes showed rapidly progressive lesions often requiring euthanasia due to severe immobility.

The earliest radiographic vertebral changes are seen mid-body, on dorso-ventral (DV) radiographs, and are bilaterally symmetric. Taxonomic differences can be seen. In non-pythonidae, square or trapezoid-shaped soft tissue densities appear on DV views cranial to the rib articulations at the level of the lateral vertebral processes. Progression involves expansion and calcification with ankyloses of vertebrae. Changes are less evident on lateral views unless severe or when proliferative changes include the dorsal aspect of the vertebral bodies. In some pythonidae, rib flaring at the spinal articulation occurs on DV views and progresses to soft tissue and bony proliferations. As lesions progress, they are also detectable on lateral radiographic views at the rib-vertebral articulations, sometimes protruding ventrally.

The etiology of these cases is still unknown. These cases were different than most spinal lesions in the literature due to the predominantly non-inflammatory histologic changes rarely reported.<sup>1-4</sup> In addition, these cases progressed rapidly and had consistent radiographic lesions. Having well-positioned whole-body radiographs and histology to differentiate osteomyelitis versus enigmatic non-inflammatory lesions has provided important insight into this ongoing investigation.

**Key words:** Non-inflammatory, osteoarthropathy, osteopathy, radiograph, snake, spinal

### ACKNOWLEDGMENTS

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**POPULATION PHARMACOKINETICS OF ENROFLOXACIN AND ITS METABOLITE CIPROFLOXACIN IN CLINICALLY DISEASED AND INJURED EASTERN BOX TURTLES (*Terrapene carolina carolina*), YELLOW-BELLIED SLIDERS (*Trachemys scripta scripta*), AND RIVER COOTERS (*Pseudemys concinna*)**

*John A. Griffioen, DVM,<sup>1,2\*</sup> Gregory A. Lewbart, MS, VMD, Dipl ACZM, DipECZM (Zoo Health Management),<sup>1</sup> Kristen Messenger, DVM, PhD, Dipl ACVAA, DACVCP,<sup>1</sup> and Mark G. Papich, DVM, DACVCP<sup>1</sup>*

<sup>1</sup>*College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27607 USA;*

<sup>2</sup>*Indianapolis Zoo, Indianapolis, IN 46222 USA*

**Abstract**

Enrofloxacin is commonly utilized for antibiotic therapy in turtles in wildlife clinics during rehabilitation. This medication is ideal for treatment due to its antibacterial activity and availability of injectable formulations; however, sufficient pharmacokinetic data to guide dosing is lacking. The objective of this study was to determine pharmacokinetic parameters of enrofloxacin and ciprofloxacin after injection of enrofloxacin in North American chelonians presenting diseased or injured to a wildlife clinic. Thirty-six Eastern box turtles (EBT, *Terrapene carolina carolina*), 23 yellow-bellied sliders (YBS, *Trachemys scripta scripta*), and 13 river cooters (RC, *Pseudemys concinna*) received a single subcutaneous injection of enrofloxacin (Baytril®, 2.27% Injectable Solution, Bayer HealthCare LLC, Shawnee Mission, KS 66201 USA) at 10 mg/kg. Each animal had blood samples collected three times between 0 and 240 hr post-injection. Pharmacokinetic parameters were determined using nonlinear mixed effects modeling (NMLE), an ideal method to allow for sparse sampling due to sampling limitations for each turtle. Results indicate overall elimination half-life ( $T_{1/2}$ ) was over 75 hr, and varied among species.  $T_{1/2}$  was 63 hr in EBT and 79 hr in YBS, longer than previously reported  $T_{1/2}$  for intracoelomic administration in healthy YBS (47.6 hr).<sup>1</sup> Variability was high for volume of distribution (steady-state) with a value of 1.4 L/kg across all samples, but 1.9 L/kg for YBS and 0.4 L/kg for RC when evaluating individual species. Antibiotic concentrations were above a minimum inhibitory concentration (MIC) value of 0.5 µg/ml for over 200 hr. These results indicate variable pharmacokinetic parameters for enrofloxacin among turtle species, which will help guide appropriate dosing protocols in diseased or injured turtles.

**Key words:** Eastern box turtle, enrofloxacin, river cooter, *Terrapene carolina carolina*, *Trachemys scripta scripta*, yellow-bellied slider

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## USE OF TRANSCUTANEOUS OXYGEN MONITORING IN LOUISIANA PINE SNAKES (*Pituophis ruthveni*)

Anastasia E. Towe, BS, BA,<sup>1\*</sup> Andrew C. Cushing, BVSc, Cert AVP (ZM), Dipl ACZM,<sup>1</sup> Christopher K. Smith, DVM, Dipl ACVAA,<sup>1</sup> Edward C. Ramsay, DVM, Dipl ACZM,<sup>1</sup> Stephen Nelson, BA,<sup>2</sup> and Luca Giori, DVM, PhD<sup>1</sup>

<sup>1</sup>College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996 USA; <sup>2</sup>Zoo Knoxville, Knoxville, TN 37914 USA

### Abstract

Accurate monitoring of oxygenation in reptiles, especially snakes, is challenging for multiple reasons, but vital for assessment of physiologic state, especially during anesthesia. Traditional pulse oximetry (SpO<sub>2</sub>) is limited by the unique anatomy and physiology of reptiles, and monitoring trends is generally considered the most clinically relevant use.<sup>2</sup> Transcutaneous oxygen monitoring utilizes near-infrared spectroscopy to assess a mixture of arterial, venous, and capillary flow to measure regional oxygen saturation (rSO<sub>2</sub>).<sup>4</sup> Uses in human medicine include assessment of cerebral oxygenation during anesthesia and assessment of soft tissues, specifically during wound healing.<sup>1,3</sup> In the present study, nine Louisiana pine snakes (*Pituophis ruthveni*) were monitored through four phases of an anesthetic event; breathing room air, supplied with supplemental oxygen, during sedation, and recovery. rSO<sub>2</sub>, measured over both the liver (rSO<sub>2</sub><sup>Liver</sup>) and at the midpoint of the body (rSO<sub>2</sub><sup>Half</sup>), and SpO<sub>2</sub>, were compared to the venous partial pressure of oxygen (PvO<sub>2</sub>) at the end of each phase. Strong positive associations were found between PvO<sub>2</sub> and both rSO<sub>2</sub><sup>Liver</sup> (r = 0.72) and rSO<sub>2</sub><sup>Half</sup> (r = 0.63) irrespective of phase, but not with SpO<sub>2</sub> (r = 0.3). Strength of correlation varied with each phase but was universally strongest for rSO<sub>2</sub><sup>Liver</sup>. Strengths of associations were further increased when rSO<sub>2</sub><sup>Liver</sup> values of < 60% were excluded from analyses. The use of rSO<sub>2</sub> in snakes appears to have merit in assessing oxygenation levels of snakes, particularly when compared to traditional pulse oximetry.

**Key words:** Blood gas, Louisiana pine snake, *Pituophis ruthveni*, transcutaneous oxygenation

### ACKNOWLEDGMENTS

The authors would like to thank the herpetology staff of Zoo Knoxville, in particular Michael Ogle. In addition, statistical assistance from Joshua Price was appreciated, and Mitch Sandifer at Masimo corporation for the use of the equipment.

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## PREVALENCE OF SNAKE FUNGAL DISEASE AT A REHABILITATED SITE (LANDFILL) AND UNDISTURBED SITE (FOREST) IN EAST TENNESSEE

Megan Gramhofer, BS,<sup>1\*</sup> Matthew C. Allender, DVM, MS, PhD, Dipl ACZM,<sup>1</sup> Ellen Haynes, DVM,<sup>1</sup> Kelsey Waterson, Diploma,<sup>2</sup> and John Byrd, MS<sup>2</sup>

<sup>1</sup>Wildlife Epidemiology Laboratory, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 USA; <sup>2</sup>Clinch River Environmental Science Organization, Oak Ridge, TN 37748 USA

### Abstract

Ophidiomycosis (snake fungal disease; SFD) is a disease of conservation concern caused by the fungus *Ophidiomyces ophiodiicola* and threatens the health of snake populations in the United States. Gaps exist in the understanding regarding the prevalence of this disease across landscapes. This study aimed to compare the prevalence of ophidiomycosis between an undisturbed forest site ( $n = 93$ ) and a rehabilitated site (reclaimed landfill;  $n = 53$ ) in Oak Ridge, TN. Snakes were examined for the presence of lesions consistent with ophidiomycosis and swabbed to detect *O. ophiodiicola* DNA using quantitative PCR. A swab of the entire body surface was collected, and an additional lesion swab was collected if lesions were observed. Snakes were released within 24 hr of capture at the site where they were found. Apparent ophidiomycosis (qPCR positive and clinical signs) was confirmed at both sites, but there was no significant difference in prevalence between the two sites (24.7% at the undisturbed site; 22.6% at the rehabilitated site). Ophidiomycosis was most prevalent in rat snakes, *Pantherophis obsoletus*, (38%) and ringneck snakes, *Diadophis punctatus*, (26%) and least prevalent in smooth earthsnakes, *Virginia valeriae*, and watersnakes, *Nerodia sipedon*, (0%). There was no difference in ophidiomycosis status between sites for the most commonly sampled species: racers, *Coluber constrictor*, rat snakes, *Pantherophis obsoletus*, or ringneck snakes, *Diadophis punctatus*. This represents the first report of ophidiomycosis in these sites in Tennessee. The findings indicate that either *O. ophiodiicola* is ubiquitous across the landscape or habitat rehabilitation efforts have achieved a similar plane of quality as native habitat.

**Key words:** *Ophidiomyces ophiodiicola*, snake fungal disease, Tennessee

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**COMPARISON OF KETAMINE-DEXMEDETOMIDINE-MIDAZOLAM VERSUS ALFAXALONE-DEXMEDETOMIDINE-MIDAZOLAM ADMINISTERED INTRAVENOUSLY TO AMERICAN ALLIGATORS (*Alligator mississippiensis*)**

*Jessica Aymen, DVM,<sup>1\*</sup> Patricia Queiroz-Williams, DVM, MS,<sup>2</sup> Chin-Chi Liu, MS, MAppStat, PhD,<sup>2</sup> and Javier G. Nevarez, DVM, PhD, Dipl ACZM, DipECZM (Herpetology)<sup>2</sup>*

<sup>1</sup>*Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON, N1G 2W1 Canada;* <sup>2</sup>*Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 USA*

**Abstract**

There are few anesthetic protocols for American alligators reported in the literature. Study objective was to compare two injectable anesthetic protocols in subadult American alligators (*Alligator mississippiensis*). Mean  $\pm$  SD body weight and length were  $4.75 \pm 0.48$  kg and  $111.52 \pm 9.93$  cm. Six clinically healthy alligators were anesthetized in a prospective randomized crossover study design. Treatments were: K (ketamine 5 mg/kg, dexmedetomidine 50  $\mu$ g/kg, and midazolam 1 mg/kg) and A (alfaxalone 5 mg/kg, dexmedetomidine 50  $\mu$ g/kg, and midazolam 1 mg/kg); both administered intravenously in the lateral occipital sinus. Anesthesia was monitored throughout its duration of 150 min. Time to induction, loss and return of reflexes (righting, withdrawal, palpebral, corneal, and cloacal), ventilation loss, and recovery were recorded. Heart rate and cardiac rhythm, respiratory rate, end-tidal carbon dioxide, esophageal and cloacal temperature were also recorded. There were no statistical differences in heart and respiratory rate, end-tidal carbon dioxide, and esophageal temperature between treatments. Palpebral, cloacal, and righting reflexes were consistently lost in all animals for both treatments. All animals recovered for both treatments. Five alligators from treatment K did not lose spontaneous ventilation ( $P = 0.0156$ ), or front and hind toe pinch withdrawal reflexes ( $P = 0.0313$ , and  $0.0313$ , respectively). In treatment A, all alligators lost ventilation, clinically significant in one individual, and front and hind toe pinch withdrawal reflexes. Alligators in treatment A were manually ventilated using room air until return of spontaneous ventilation. Consequently, at the doses used and given intravenously, K treatment caused less apnea than treatment A.

**Key words:** Alfaxalone, alligator, *Alligator mississippiensis*, anesthesia, ketamine, reflexes

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## THE QUEST FOR A RELIABLE BIOMARKER OF IRON OVERLOAD DISORDER IN THE RHINOCEROS

*Terri L. Roth, MS, PhD*

*Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH 45220 USA*

### Abstract

It is well documented that zoo-managed black rhinoceroses (*Diceros bicornis*) and Sumatran rhinoceroses (*Dicerorhinus sumatrensis*) store excessive iron as hemosiderosis in body organ tissue, especially that of the liver;<sup>3</sup> however, the etiology of iron storage and the extent of its impact on rhinoceros health are obscure and likely complex. Recent research has revealed serum ferritin concentration is unreliable as a measure of iron overload disorder (IOD) severity in the rhinoceros.<sup>4,5</sup> Therefore, new diagnostic biomarkers are being pursued.

Hyaluronic acid (HA) is rapidly filtered by the liver of healthy individuals, and serum concentrations increase when the liver is compromised.<sup>2</sup> Two HA assays (Echelon Biosciences Inc., 675 Arapeen Dr., Suite 302 Salt Lake City, UT, 84108 USA [product number K-1200] and Corgenix, 11575 Main Street Suite 400, Broomfield, CO, 80020 USA [product number 029-001]) were employed for testing Sumatran rhinoceros serum samples ( $n = 71$ ). Although concentrations differed between assays, results were highly correlated ( $r = 0.92$ ;  $P < 0.01$ ). Hyaluronic acid concentrations were elevated in symptomatic individuals sick with IOD (Echelon assay values, mean  $\pm$  SD;  $1,084 \pm 359$  ng HA/ml) compared to those in both the same individuals when asymptomatic ( $278 \pm 174$  ng HA/ml) and another sick Sumatran rhinoceros ( $157 \pm 40$  ng HA/ml) with high serum ferritin concentrations ( $4,927 \pm 1,019$  ng/ml) that died of thyroid cancer with only mild hemosiderosis. In contrast, HA concentrations were not markedly elevated in samples from any of the eight outwardly healthy black rhinoceroses tested ( $180 \pm 60$  ng HA/ml; range, 119-280 ng HA/ml) despite the wide range in serum ferritin concentrations among individuals (140-127,891 ng/ml).

Under iron overload conditions, serum can contain non-transferrin bound iron, a fraction of which may be labile plasma iron (LPI). LPI is especially damaging because it is redox active, capable of permeating into organs and catalyzing the formation of reactive oxygen species (ROS) in tissues.<sup>1</sup> An LPI assay (Aferrix Ltd., Kiryat Atidim, POB 58129, Tel-Aviv 61581, Israel, FeROS™ LPI kit, product reference: TSL902 V.12) that relies on a selective iron chelator to quantify iron-mediated ROS generation was used to test rhino serum. Among Sumatran rhinoceros samples ( $n = 19$  samples from seven rhinoceroses), only those from symptomatic individuals sick with IOD tested positive for LPI; however, data for black rhinoceroses ( $n = 16$  samples from six rhinoceroses) were inconsistent. Black rhinoceroses that were LPI-positive were not always clinically ill, nor did they die within 3 yr of sampling. Interestingly, even in some LPI-negative black rhinoceros samples, ROS activity was substantial, suggesting another redox active agent (other than iron) may be present in the serum. In contrast, the ROS reaction was minimal in all Sumatran rhinoceros serum samples, even those with positive LPI values. Follow-up analyses are underway to identify the cause of the excessive ROS activity observed in black rhinoceros serum.

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These preliminary data suggest that 1) HA may be useful in diagnosing end-stage liver failure in rhinoceroses, 2) LPI may be an accurate indicator of end-stage IOD in Sumatran rhinoceroses, 3) test results differ between black rhinoceroses and Sumatran rhinoceroses, and 4) serum from some black rhinoceroses appears highly redox active even in the presence of an iron-specific chelator. Overall, results offer insight into alternative biomarkers that may prove useful for diagnosing rhinoceros IOD or liver failure. Furthermore, inter-specific variability in the results suggests that iron dysfunction may differ between the Sumatran rhinoceros and the black rhinoceros.

**Key words:** Black rhinoceros, hyaluronic acid, iron overload, labile plasma iron, reactive oxygen species, Sumatran rhinoceros

#### ACKNOWLEDGMENTS

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## URINE CRYSTALS CORRELATED WITH TWO CASES OF PATHOGENIC UROLITHIASIS IN ASIAN ELEPHANTS (*Elephas maximus*)

*Lydia Young, DVM,\* Eileen Keating, RVT, VTS, ECC, and Lauri Crimmins, DVM*

*The Elephant Sanctuary, Hohenwald, TN 38462 USA*

### Abstract

Crystalluria is a common finding in Asian elephants, but urethral calculi and uroliths are infrequently reported.<sup>2,3</sup> Parameters have not been established to determine if urine crystals indicate pathogenesis.<sup>1</sup> In this study, 91 urine samples from 5 female Asian elephants were retrospectively analyzed for crystal occurrence and type. Overall, 79% of samples contained crystals. Types identified were calcium carbonate (58%), calcium oxalate (17%), struvite (17%), and amorphous (58%). Two cases of urolithiasis were diagnosed postmortem. Case 1, a 49-yr-old, had a 6.9-kg urolith (27.9 cm × 12.7 cm). Superficial urolith analysis was 100% calcium carbonate. Of 25 urine samples from case 1, 15 contained crystals; 87% were calcium carbonate. Case 2, a 53-yr-old, had a 10.7-kg urolith (29 cm × 23 cm). External analysis, sectioning, faxitron scan, spectral analysis, and CT scan were performed at the Minnesota Urolith Center. Stone composition was 100% calcium carbonate. Of 36 urine samples from case 2, 30 contained crystals; 96% were calcium carbonate. No significant differences between type, frequency, and quantity of crystals were present between urolith and non-urolith groups, indicating no predictive value between presence of urine crystals and pathogenic urolithiasis; however, in the urolith group, there was a correlation between crystalluria type and urolith composition, indicating urine crystal type may be a useful indicator of stone composition if one is identified via diagnostic imaging.

**Key words:** Crystalluria, *Elephas maximus*, urolith

### ACKNOWLEDGMENTS

The authors would like to acknowledge the Minnesota Urolith Center for performing urolith analysis, Dr. Steven Scott, Director of Veterinary Care, for his compassionate, dedicated service to The Sanctuary's elephants since 1995, and the elephant caregivers, whose excellent daily care makes individualized veterinary care possible.

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## A NOVEL TREATMENT FOR SUBSOLAR AND DIGITAL ABSCESES UTILIZING AN EPOXY PATCH SYSTEM PAIRED WITH ANTIMICROBIAL WOUND SPONGES IN ASIAN ELEPHANTS (*Elephas maximus*)

Lydia Young, DVM\* and Eileen Keating, RVT, VTS, ECC

The Elephant Sanctuary, Hohenwald, TN 38462 USA

### Abstract

Foot abscesses are reportedly the single most significant ailment of Asian elephants (*Elephas maximus*) in captivity.<sup>1</sup> Conventional treatment methods recommend debridement of all affected tissue, soaking, and topically applied medications.<sup>1</sup> Protective boots and wraps have been used with limited success.<sup>1,3</sup> In this study, five chronic foot abscesses in female Asian elephants were successfully treated with a novel therapeutic approach, resulting in full resolution in 4/5 cases (Table 1).

Treatment steps include cryotherapy-assisted (Kendall AMD foam dressing, Covidien Laboratories Medtronic Minneapolis 55432 USA) debridement of necrotic tissue, placement of an antimicrobial sponge (Verruca Freeze cryosurgery spray CryoSurgery, Inc. Nashville TN 37205 USA) in the abscess tract, application of epoxy products (Vettec Adhere Equi-thane, Vettec Inc., Orange, CA 92867 USA and Vettec Sole-Guard, Vettec Inc., Orange, CA 92867 USA) on a rubber patch (i.e., recycled vehicle inner tube) over the affected area, and a second epoxy coating. All animals in the study showed immediate improvement in ambulation following patch application. Patches were maintained for 4-7 days and replaced immediately upon removal. The ease of application, continuous tissue protection, antimicrobial delivery, and increase in comfort make it an excellent option for treatment in challenging foot abscess cases.

**Key words:** Abscess, digital, *Elephas maximus*, epoxy, subsolar

### ACKNOWLEDGMENTS

The authors thank Dr. Steven Scott, Director of Veterinary Care at The Elephant Sanctuary in Tennessee, for his compassionate, dedicated service to elephants since 1995, and the elephant caregivers, whose excellent daily work makes individualized veterinary care possible.

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**Table 1.** Chronic foot abscess treatment and resolution in five female Asian elephants.

Signalment	Abscess Location	Initial abscess size (diameter)	Duration prior to epoxy patch	Resolution time with epoxy patch therapy
70 yr	RF sole	10 cm	18 mo	13 mo
70 yr	LF sole	12 cm	20 mo	15 mo
50 yr	RF digit 3	3 cm	5+ yr	4 mo
50 yr	LF digit 3	3.5 cm	4 yr	3.5 mo
46 yr	RF digit 4	4 cm	5 + yr	Ongoing

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# ANATOMIC STUDY OF THE ASIAN ELEPHANT (*Elephas maximus*) MANUS USING DIGITAL IMAGERY OF COMPUTED TOMOGRAPHY AND MAGNETIC RESONANCE IMAGERY AS COMPARED TO EPOXY-SHEET PLASTINATION PRESERVATION TECHNIQUE

*Paul B. Nader, DVM\* and Robert W. Henry, DVM, PhD*

*Lincoln Memorial University, College of Veterinary Medicine, Harrogate, TN 37752 USA*

## Abstract

The Asian elephant, *Elephas maximus*, has served as a companion species, beast of burden, zoological icon, and charismatic species for centuries by humans. It is incumbent for all human caretakers of this iconic mega-vertebrate to maximize their knowledge of this species and incorporate that knowledge into the daily care and benefit of this species. This gross anatomic study of the soft-tissue and osteologic tissues of the Asian elephant manus strives to increase and expand the scientific knowledge of the inter-relationships of the key soft-tissues, including skeletal muscle, tendons, ligaments, joint capsules, and major blood vessels with their osteologic components.<sup>1,2</sup> Using the advanced digital imagery of both computed tomography and magnetic resonance imagery and advanced three-dimensional, computer-modelling, the specialized architecture of the elephant manus can more easily be illustrated and comprehended. In addition, with better imagery, the veterinary clinician can better detect and diagnose pathologies within either the soft-tissues or osteologic tissues within the elephant manus. Once all digital imagery was completed, the actual cross-sectional specimens of the elephant's study specimen, both manus and distal antebrachium, were preserved for decades into the future via epoxy-sheet plastination technique. This anatomic study displays the valuable visual benefits of advanced digital imagery and its validity was confirmed when compared to the plastinated, cross-sectional specimens of the Asian elephant manus.

**Key words:** Asian elephant, computer tomography, *Elephas maximus*, epoxy-sheet plastination, magnetic resonance imagery, manus

## ACKNOWLEDGMENTS

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## ORAL PROGESTIN TREATMENT FACILITATES IMPROVED REPRODUCTIVE MANAGEMENT OF WILD FELIDS

*William F. Swanson, DVM, PhD,<sup>1\*</sup> Raquel Gonzalez Herrero, DVM, PhD,<sup>1</sup> Amy Miller, MS,<sup>1</sup> Ginger Sturgeon, DVM,<sup>2</sup> Alicia Hahn, DVM, Dipl ACZM,<sup>2</sup> Jennifer D'Agostino, DVM, Dipl ACZM,<sup>3</sup> Gretchen A. Cole, DVM, Dipl ACZM, DipECZM (Zoo Health Management),<sup>3</sup> and Lindsey M. Vansandt, DVM, PhD<sup>1</sup>*

<sup>1</sup>*Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH 45220 USA;* <sup>2</sup>*Pittsburgh Zoo & PPG Aquarium, Pittsburgh PA 15206 USA;* <sup>3</sup>*Oklahoma City Zoological Park, Oklahoma City, OK 73111 USA*

### Abstract

Progesterone has myriad effects on the female cat's reproductive system, all ultimately geared toward supporting embryo and fetal development from conception to parturition. In this role, progesterone also acts via feedback loops on the hypothalamus and pituitary gland to suppress ovarian follicular growth and estrogen production. These actions of endogenous progesterone can be mimicked by synthetic progestins, either administered parenterally, as depot injections or implants (e.g., for long-term contraception), or orally intermixed with daily diets. The primary advantage of oral delivery is ease of administration and rapid reversal on withdrawal. Oral progestin treatment has proven valuable for improving reproductive management of felid species as shown by the three following examples. First, oral administration of the progestin altrenogest (Regumate, Merck Animal Health, Madison, NJ 07940 USA), is highly effective for suppressing ovarian activity in domestic cats prior to exogenous gonadotropin treatment for assisted reproduction.<sup>1</sup> Our extrapolation of this approach to wild felids for fixed time laparoscopic oviductal artificial insemination (LO-AI) has resulted in multiple pregnancies in four small cat species (fishing cat, *Prionailurus viverrinus*; ocelot, *Leopardus pardalis*; Pallas' cat, *Otocolobus manul*; sand cat, *Felis margarita*).<sup>2</sup> In our most recent LO-AI procedures in ocelots, three ovulatory females were inseminated following altrenogest-induced suppression (0.044 mg/kg BW; 30 days), 7 days of progestin withdrawal and gonadotropin treatment. Based on fecal progesterone analyses, two females appeared to conceive following LO-AI with frozen semen and subsequently gave birth to three viable kittens after 82 days of gestation. The second example involves the use of oral progestin as therapy for a 12-yr-old ocelot with suspected ovarian cysts. This proven founder female demonstrated persistent estrual phases that failed to abate following repeated breeding by the male, and ultrasonography revealed multiple cystic follicles on her ovaries. Daily oral treatment with altrenogest (0.044 mg/kg BW, 30 days) was implemented to suppress follicular growth and attempt to reset normal ovarian function. Two weeks following altrenogest cessation, the female returned to estrus, bred with the male and conceived, producing a healthy male kitten after an 81-day gestation. The third example involves oral progestin treatment for possible recurrent pregnancy loss in a 12-yr-old Amur leopard (*Panthera pardus orientalis*). This female experienced fetal loss due to dystocia at the end of her first pregnancy and then, over the next 2 yr, bred repeatedly with the male during multiple estrus phases. Although this leopard exhibited periods of acyclicity and/or elevated fecal progesterone consistent with induced ovulation and possible pregnancy, no offspring were produced. Occasional blood spotting 1-2 mo post-breeding supported the possibility of early pregnancy loss. Following one period of observed breeding,

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abdominal ultrasonography conducted 45 days later confirmed the presence of a developing fetus. The female was fed altrenogest daily (0.176 mg/kg BW) from day 50 to day 90 of pregnancy and then gradually weaned from progestin over the next 3 days. At day 99, the female went into labor and gave birth to a healthy female kitten, her first viable offspring. Our results indicate that oral progestin treatment is an effective method of ovarian suppression to precisely synchronize ocelots and other wild felids for successful fixed time LO-AI procedures. Knowledge of physiologically relevant progestin dosages gained from AI studies was directly applicable to treating suspected ovarian cysts in a founder ocelot, and for providing hormonal support of pregnancy in an aging nulliparous Amur leopard. These latter case reports provide evidence that oral progestin treatment may have further value therapeutically for correcting reproductive abnormalities in wild felids for improved reproductive management.

**Key words:** Altrenogest, artificial insemination, ovarian cysts, pregnancy loss, progesterone

#### **ACKNOWLEDGMENTS**

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## COMPARISON OF DIAGNOSTIC PREDICTORS OF NEONATAL VIABILITY IN NONDOMESTIC CAPRINAE AT THE SAN DIEGO ZOO AND THE SAN DIEGO ZOO SAFARI PARK

*Taylor N. Bliss, DVM,<sup>1\*</sup> Matt J. Marinkovich, DVM,<sup>2</sup> Rachel E. Burns, DVM, DACVP,<sup>3</sup> Meredith M. Clancy, DVM, MPH, Dipl ACZM,<sup>2</sup> and Lauren L. Howard, DVM, Dipl ACZM<sup>2</sup>*

*<sup>1</sup>VCA Emergency Animal Hospital & Referral Center, San Diego, CA 92108 USA; <sup>2</sup>San Diego Zoo Global, San Diego Safari Park, Escondido, CA 92027 USA; <sup>3</sup>San Diego Zoo Institute for Conservation Research, Escondido, CA 92027 USA*

### Abstract

Ruminant neonatal survival is reliant upon an adequate amount of immunoglobulin consumption within a specific time frame post parturition.<sup>1-5</sup> Failure of passive transfer (FPT) of immunoglobulin has the potential to increase morbidity and mortality in neonates.<sup>1,2</sup> Various methods to identify cases of FPT in nondomestic Caprinae species have been utilized, but their diagnostic value is poorly understood.<sup>2</sup> This retrospective study evaluated 5 methods to assess passive transfer status in nondomestic Caprinae species in a zoological collection. A total of 187 neonates from eleven nondomestic Caprinae species had one or more testing methods performed within 7 days of birth: glutaraldehyde coagulation test ( $n = 144$ ), serum gamma-glutamyltransferase ( $n = 170$ ), glucose ( $n = 163$ ), fibrinogen ( $n = 162$ ) and BOVA-S, sodium sulfite turbidity test ( $n = 41$ ). Positive and negative results of each test were compared with the clinical condition (alive or dead) at 7 days of age using Fisher's exact test single variable analysis for the entire population and individual species groups. Data analysis indicates the only statistically significant predictor of survival to 7 days in the population was a blood glucose  $\geq 60$  mg/dl ( $P = 0.014$ ). Multi-variable analyses, larger sample sizes among sub-groups, or longer follow-up may provide better evaluation of FPT testing methods as predictors of survival. The use of multiple testing methods to determine neonatal viability in nondomestic Caprinae species is warranted as no one method was adequate for identifying FPT.

**Key words:** Caprinae, failure of passive transfer, glutaraldehyde coagulation, neonate, passive immunity, ruminant

### ACKNOWLEDGMENTS

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## OVARIAN SYNCHRONIZATION, OVULATION INDUCTION, AND SUCCESSFUL ARTIFICIAL INSEMINATION IN THE JAGUAR (*Panthera onca*)

Lindsey Marie Vansandt, DVM, PhD,<sup>1\*</sup> Cristina Harumi Adania, DVM, PhD,<sup>2</sup> Priscila Rocha Yanai, DVM,<sup>2</sup> Jéssica da Silva Paulino, DVM,<sup>2</sup> Regina Célia Rodrigues da Paz, DVM, PhD,<sup>3</sup> Helen L. Bateman, MS,<sup>1</sup> and William F. Swanson, DVM, PhD<sup>1</sup>

<sup>1</sup>Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, Cincinnati, OH 45220 USA; <sup>2</sup>Centro Brasileiro para Conservação dos Felinos Neotropicais, Associação Mata Ciliar, Jundiá, SP 13212-010 Brazil; <sup>3</sup>Laboratório de Pesquisa em Animais de Zoológico, Faculdade de Medicina Veterinária, Universidade Federal de Mato Grosso, Cuiabá, MT 78060-900 Brazil

### Abstract

Within zoological institutions, there is a growing realization that assisted reproductive technologies (ART) will be necessary for genetic management and long-term sustainability of imperiled felids.<sup>1</sup> Semen banking and artificial insemination (AI) are the most readily applicable ART for management of felid populations, but differences in physiology necessitate fundamental research in each felid species before the potential of ART can be realized. The jaguar (*Panthera onca*) is the largest wild cat native to the Americas and a focal species for conservation efforts, but their reproductive biology is poorly understood and a successful jaguar AI has never been reported. Accordingly, our objectives in this study were to (1) assess ovarian sensitivity to oral progestin, (2) evaluate ovarian responses following gonadotropin treatment, and (3) investigate fertility with fixed time laparoscopic oviductal AI. Adult female jaguars were fed oral progestin (Altrenogest, Regu-Mate<sup>®</sup>, Merck Animal Health, Summit, NJ, 07901; 0.044 mg/kg  $n = 2$ ; 0.088 mg/kg  $n = 2$ ) daily for 45 days. Longitudinal fecal hormone analyses revealed that neither altrenogest dose was sufficient to suppress ovarian activity. Therefore, the progestin dosage was increased for Objectives 2 and 3. Females were fed altrenogest (0.088 mg/kg,  $n = 4$ ; 0.176 mg/kg,  $n = 4$ ) for 45-48 days, and following a 5-day withdrawal period, treated with exogenous gonadotropins: 600 IU equine chorionic gonadotropin (eCG) followed 82 hr later by 5000 IU porcine luteinizing hormone (pLH). At 43-48 hr post-pLH, females were evaluated laparoscopically for ovarian response, and inseminated in each oviduct with fresh semen ( $1.5-4.2 \times 10^6$  motile sperm/oviduct). If the oviduct could not be cannulated ( $n = 5$  oviducts total), fresh semen was deposited into the ipsilateral uterine horn ( $5-12.6 \times 10^6$  motile sperm/horn). All females produced multiple follicles in response to gonadotropin treatment ( $21.3 \pm 4.2$  follicles plus corpora lutea (CL); mean  $\pm$  SE), but ovulatory rate was low ( $18.7 \pm 9.3\%$  of follicles) and females averaged only  $3.6 \pm 2.0$  CL. No births resulted from the AIs. For subsequent AI procedures, three adjustments were made to the hormone treatment protocol: (1) the progestin withdrawal period was increased to 7 days, (2) eCG-pLH interval was increased to 90-92 hr, and (3) pLH dose was increased to 10,000 IU. Females ( $n = 5$ ) all received the high progestin dose (0.176 mg/kg). At laparoscopy, all females had multiple ovulations with  $57.1 \pm 6.9\%$  of follicles ovulating, forming  $14.2 \pm 2.3$  CL per female. Females were inseminated laparoscopically in each oviduct with fresh semen ( $2.5-4.4 \times 10^6$  motile sperm/oviduct) except in two cases the oviduct could not be cannulated and  $5-7.5 \times 10^6$  motile sperm was deposited into the ipsilateral uterine horn. One of the five females conceived and, after a 104-day gestation period, gave birth to a single viable cub, the first jaguar ever produced from

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AI. In summary, oral progestin and eCG/pLH gonadotropin treatment, following species-specific adjustments, proved to be effective for ovarian synchronization in the jaguar, providing a consistent and robust ovarian response. The birth of a live cub following AI further confirms the effectiveness of the hormone regimen and provides an important milestone towards the application of ART for population management of this imperiled felid.

**Key words:** Altrenogest, artificial insemination, assisted reproductive technologies, equine chorionic gonadotropin, jaguar, *Panthera onca*, porcine luteinizing hormone

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## CHARACTERIZATION OF 13,14-DIHYDRO-15-KETO-PROSTAGLANDIN F2-ALPHA METABOLITE IN THE PALLAS' CAT (*Otocolobus manul*) AND SAND CAT (*Felis margarita*) AS A NON-INVASIVE MARKER FOR PREGNANCY

Lydia Margaret Hall, BS,<sup>1,2\*</sup> Amy Miller, MS,<sup>2</sup> Sandy L. Fritz, MATLT,<sup>2</sup> William F. Swanson, DVM, PhD,<sup>2</sup> and Lindsey Marie Vansandt, DVM, PhD<sup>2</sup>

<sup>1</sup>Purdue University College of Veterinary Medicine, West Lafayette, IN 47907 USA; <sup>2</sup>Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, Cincinnati, OH 45220 USA

### Abstract

Nearly half of the species in the family Felidae are included on the IUCN Red List of Threatened Species.<sup>5</sup> As wild populations dwindle, cats housed in zoos are becoming ever more critical for conservation by serving as assurance populations to protect against extinction. Due to a combination of poor breeding success, low founder numbers, and small population sizes, the long-term sustainability of most felid populations in North American zoos cannot be achieved with natural breeding alone. The development of assisted reproductive technologies (ART) may be critical to ensure their future viability.<sup>7</sup> Non-invasive fecal hormone metabolite monitoring has provided these breeding programs with a pregnancy diagnostic tool; however, for many felid species, increase in progesterone during a non-pregnant luteal phase is indistinguishable from that of pregnancy, and non-pregnant luteal phases can last up to two-thirds the duration of gestation, depending on the species. For example, pregnancy lasts 66-77 days in the Pallas' cat (*Otocolobus manul*), while their non-pregnant luteal phase is ~50 days, restricting accurate pregnancy diagnosis to just 20 days before parturition.<sup>1</sup> This delay in pregnancy diagnosis to the latter part of gestation creates uncertainty in planning for parturition and neonatal care. Moreover, fecal progesterone monitoring cannot distinguish between post-implantation pregnancy loss and conception failure, which is relevant for assessing both natural breeding and AI success. Because of these challenges, recent research has focused on investigating alternative pregnancy markers to improve the efficiency and accuracy of non-invasive pregnancy diagnosis. One promising pregnancy marker is 13,14-dihydro-15-keto-prostaglandin F2alpha metabolite (PGFM), a stable fecal metabolite generated during corpora lutea lysis.<sup>6</sup> A pronounced spike in PGFM occurring only in pregnant individuals during the last trimester has been observed reliably in 10 of the 14 *Felidae* genera (*Acinonyx*, *Caracal*, *Catopuma*, *Felis*, *Leopardus*, *Leptailurus*, *Lynx*, *Neofelis*, *Prionailurus*, *Puma*), with variation in the presence of a characteristic peak in one genus (*Panthera*).<sup>2,4</sup> In this study, our objectives were to 1) characterize fecal PGFM concentrations during the non-pregnant luteal phase and pregnancy in Pallas' cats, one of the last uncharacterized genera, *Otocolobus*, and 2) further investigate fecal PGFM levels in pregnant and luteal-phase sand cats (*Felis margarita*), using a commercially available PGFM enzyme linked immunoassay (PGFM EIA Kit, Arbor Assays®, Ann Arbor, MI 48108-3284 USA). A previous study reported initial characterization of PGFM for one pregnant and one non-pregnant luteal phase sand cat, finding definitive differences in PGFM levels.<sup>2</sup> In our study, fecal PGFM concentrations were assessed in pregnant Pallas' cats (natural mating,  $n = 3$ ; AI,  $n = 2$ ) from samples collected beginning 85 days before parturition until 0-1 days after parturition. For non-pregnant Pallas' cats (natural mating,  $n = 3$ ; AI,  $n = 2$ ), samples were assayed in each female for 79-85 days, beginning 9-14 days before ovulation and initial

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progesterone increase. In sand cats (natural breeding, pregnant,  $n = 3$ ; AI, pregnant,  $n = 1$ ; natural breeding, non-pregnant,  $n = 2$ ; AI, non-pregnant,  $n = 2$ ), a similar fecal sampling schedule was used to assess PGFM concentrations. Data are presented as means  $\pm$  standard error (SE), and mean values were compared using Student's  $t$ -test. Basal PGFM values were calculated using a two standard deviation (SD) iterative process; an elevation of PGFM, in three consecutive samples, that was greater than the baseline value plus two SDs was considered a substantive increase.<sup>3</sup> In pregnant Pallas' cats, fecal PGFM remained basal ( $1526.4 \pm 260.3$  ng/g) until 29-35 days before birth. PGFM concentrations subsequently exhibited an initial increase ( $5439.2 \pm 1259.4$  ng/g) above ( $P = 0.02$ ) baseline levels, and continued increasing steadily, with maximum values ( $132068.3 \pm 38,105.7$  ng/g) observed 0-9 days before parturition. In contrast, PGFM levels in the five non-pregnant Pallas' cats remained near baseline ( $1250.5 \pm 165.0$  ng/g) throughout their luteal phase, with no increase ( $P = 0.99$ ) in values ( $1247.8 \pm 305.8$  ng/g) observed in the 39-43 day time period after initial progesterone elevation (i.e., corresponding to the time frame observed for the initial PGFM rise in pregnant Pallas' cats). Notably, one Pallas' cat that was artificially inseminated and categorized as non-pregnant showed a sharp increase in PGFM ( $39619.7$  ng/g) at 51 days post-AI; values remained elevated for 4 days before steadily declining to baseline 67 days post-AI. These findings suggest that this female did conceive but lost the pregnancy several weeks before full-term gestation. In pregnant sand cats, the timing of the initial PGFM increase (2-27 days before parturition) and associated PGFM levels ( $8415.8 \pm 3745.8$  ng/g) were highly variable, and did not differ ( $P = 0.10$ ) from baseline values ( $1888.8 \pm 950.8$ ). Following the initial PGFM rise, maximum values ( $30571.0 \pm 8471.5$  ng/g) that were higher ( $P = 0.04$ ) than baseline occurred 2-9 days before parturition. PGFM concentrations ( $520.0 \pm 62.7$  ng/g) in the four non-pregnant sand cats remained ( $P = 0.78$ ) near baseline ( $552.9 \pm 122.7$  ng/g) throughout the sampling period. In conclusion, our findings have shown that PGFM analysis is a reliable diagnostic tool for pregnancy in Pallas' cats, and confirm its utility for pregnancy diagnosis in sand cats, albeit much closer to the time of parturition. In Pallas' cats, the initial increase in PGFM occurs  $\sim$ 1 mo before birth (or 9-15 days earlier than the progesterone decline in a non-pregnant luteal phase) and remains elevated until parturition. In sand cats, the timing of this initial diagnostic PGFM increase is much more variable, occurring from 2 to 27 days before birth. In both species, these PGFM increases appear to be highly specific to pregnancy since similar elevations do not occur during non-pregnant luteal phases. This diagnostic tool is valuable to curators and population managers by allowing earlier confirmation of pregnancy, as well as identification of females that possibly conceive but fail to carry offspring to term.

**Key words:** *Felis margarita*, non-pregnant luteal phase, *Otocolobus manul*, Pallas' cat, PGFM enzyme linked immune assay, pregnancy detection, prostaglandin F2 $\alpha$  metabolite, sand cat

#### ACKNOWLEDGMENTS

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## HISTOPATHOLOGIC EVIDENCE OF SPONTANEOUS OVULATION IN TIGERS (*Panthera tigris*)

Megan L. Cabot, DVM,<sup>1\*</sup> Edward C. Ramsay, DVM, Dipl ACZM,<sup>1</sup> Deborah Chaffins,<sup>3</sup> and Mee-Ja M. Sula, DVM, DACVP<sup>2</sup>

<sup>1</sup>Departments of Small Animal Clinical Sciences and <sup>2</sup>Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996 USA; <sup>3</sup>Tiger Haven, Inc., Kingston, TN 37763 USA

### Abstract

Spontaneous ovulation has been identified in several wild felid species, as well as in domestic cats, previously thought to undergo only induced ovulation.<sup>1,3,4</sup> Limited studies have assessed ovulation patterns in tigers (*Panthera tigris*) and those have not found evidence of spontaneous ovulation in this species;<sup>2,6</sup> however, uterine pathology typically associated with prolonged progesterone exposure has been identified in unbred tigers, suggesting spontaneous ovulation is occurring.<sup>5</sup> Ovaries from 47 tigers, previously submitted for histopathology, were reviewed. The presence or lack of corpora lutea (CL) was documented and compared with social housing condition for each animal. Social housing categories were: female housed alone; female housed with other females; female housed with one or more castrated males; and female housed with one or more intact males. Active CL were identified in 66% (10/15) of females housed alone, 85% (6/7) of females housed with other females, and 58% of females housed with a castrated male. The only female housed with an intact male did not have active CL. A chi squared test found that the presence of CL was independent of social housing condition. These results offer strong evidence of the potential for spontaneous ovulation in tigers that do not experience contact and/or breeding. This finding could impact assisted reproduction efforts as it suggests a luteal control protocol with an early luteolytic agent may have more success. It also supports spaying non-reproductive or post-reproductive female tigers to reduce the rate of uterine infections.

**Key words:** Corpora lutea, histopathology, *Panthera tigris*, spontaneous ovulation, tiger

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## A RETROSPECTIVE EVALUATION OF BINTURONG (*Arctictis binturong*) MORBIDITY AND MORTALITY UNDER MANAGED CARE WITHIN U.S. INSTITUTIONS

Alex McFarland, BA,<sup>1,2\*</sup> Anneke Moresco, MS, DVM, PhD,<sup>2</sup> and Celeste Lombardi, BS<sup>3</sup>

<sup>1</sup>Denver Zoo, Denver, CO 80205 USA; <sup>2</sup>Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523 USA; <sup>3</sup>Columbus Zoo, Columbus, OH 43065 USA

### Abstract

Limited data are available summarizing binturong (*Arctictis binturong*) morbidity and mortality. Medical records and necropsies were summarized from 16 institutions through either completion of a survey or submission of complete medical records. Responses included 44 individuals from the years 1986-2011, 18 of which had necropsy data available. Median age at death was 16.3 yr. The primary cause of mortality was neoplasia 55% (10/18) and cardiovascular disease 28% (5/18). The remaining three were euthanized due to an endocrine disorder, neurologic disorder, and a non-neoplastic renal disorder. Neoplasms included renal carcinoma/sarcoma 40% (4/10), mammary gland carcinoma 20% (2/10) as well single instances of lymphosarcoma, uterine carcinoma, chemodectoma, and an undifferentiated liver mass. Pancreatic islet cell carcinoma was also once found as a comorbidity. Diagnoses were summarized by affected organ system, a few categories are clinical signs because no diagnosis was made. Primary affected systems were gastrointestinal 20%, integumentary 16%, behavioral 14%, renal 10%, and musculoskeletal 10%. Conditions were diagnosed most commonly (54%) in young to late adult stage (1-12 yr). Although renal neoplasia and cardiovascular disease was a common cause of mortality, it was rarely diagnosed antemortem, and therefore does not appear as a common disease in the morbidity figures. It appears that binturongs commonly have subclinical renal and cardiovascular disease. Preventive medicine focused on detecting these diseases during routine physical exams may result in earlier detection. 86% (37/43) had available vaccine records with no reported reactions. Vaccines included: inactivated Rabies, canine and ferret attenuated distemper formulations and inactivated feline panleukopenia formulations.

**Key words:** *Arctictis binturong*, mammary adenocarcinoma, morbidity, mortality, neoplasia, renal carcinoma

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## CORRELATIONS BETWEEN SERUM COPPER AND LIVER COPPER LEVELS IN NONDOMESTIC BOVIDS AT THE SAN DIEGO ZOO SAFARI PARK, 2013-2018

*William R. Culver III,<sup>1\*</sup> Meredith M. Clancy, DVM, MPH, Dipl ACZM,<sup>2</sup> and Lauren L. Howard, DVM, Dipl ACZM<sup>2</sup>*

*<sup>1</sup>Population Health Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA 10460 USA; <sup>2</sup>Harter Veterinary Medical Center, San Diego Zoo Safari Park, Escondido, CA 92027 USA*

### Abstract

Copper deficiency is a concern in nondomestic bovids at the San Diego Zoo Safari Park (SDZSP) with documented deficiency in individuals noted postmortem.<sup>1,2</sup> Adequate antemortem diagnosis remains a challenge as serum copper levels may have inadequate sensitivity/specificity when compared to postmortem liver samples. Medical records were evaluated for bovids that died from 1 Jan 2013 to 15 Jul 2018 to identify those that had postmortem liver copper levels ( $n = 888$ ). This study evaluated the subset of this study population with antemortem serum copper levels collected 0-3 mo ( $n = 130$ ) and 3-6 mo ( $n = 11$ ) prior to death. Censoring animals that received copper boluses (3 mo,  $n = 53$ ; 6 mo,  $n = 57$ ), Spearman rank correlations analyzed the association between paired samples by subfamily and genus. Bovinae females showed positive significant associations between serum/liver copper, 0-3 mo prior to death ( $r = 0.72$ ,  $n = 15$ ,  $P = 0.002$ ). Caprinae of both sexes ( $r = -0.62$ ,  $n = 23$ ,  $P = 0.002$ ) and Reduncinae females ( $r = -0.79$ ,  $n = 7$ ,  $P = 0.036$ ) showed negative associations 0-3 mo prior to death. Sensitivity/specificity were still poor with positive correlations but improved with censored neonates. Subfamily variation is noted with more covariable investigation warranted to provide a clearer picture. Continued postmortem sample collection and expanded antemortem testing will better define parameters for diagnosing copper deficiency in nondomestic bovids.

**Key words:** Bovidae, captivity, liver copper levels, nondomestic, trace element serum levels

### ACKNOWLEDGMENTS

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## TIGER (*Panthera tigris*) AND DOMESTIC CAT (*Felis catus*) IMMUNE RESPONSES TO CANARYPOX-VECTORED CANINE DISTEMPER VACCINATION

Michael McEntire, DVM,<sup>1\*</sup> Edward C. Ramsay, DVM, Dipl ACZM,<sup>1</sup> Stephen Kania, PhD,<sup>2</sup> Peter Prestia, DVM,<sup>1</sup> Eman Anis, BVMS, PhD,<sup>3</sup> Andrew Cushing, BVSc, Cert AVP (ZM), Dipl ACZM,<sup>1</sup> and Rebecca P. Wilkes, DVM, PhD, Dipl ACVM<sup>3</sup>

<sup>1</sup>Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996 USA; <sup>2</sup>Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996 USA; <sup>3</sup>Veterinary Diagnostic and Investigational Laboratory, College of Veterinary Medicine, The University of Georgia, Tifton, GA 31793 USA

### Abstract

Canine distemper virus (CDV) is a significant threat to endangered wild felids.<sup>1</sup> An ideal vaccination strategy has yet to be elucidated.<sup>2-3</sup> Two methods for delivering a canarypox vectored CDV vaccine to tigers (*Panthera tigris*) and domestic cats (*Felis catus*) were investigated. Eight tigers were divided randomly into two vaccination groups: subcutaneous injection or topical tonsillar application. Each received 2 ml of CDV vaccine (PureVax Ferret Distemper Vaccine, Merial, Duluth, GA 30096, USA). Blood was collected from tigers on days 0, 21, 35 or 37, and 112 post-initial vaccination (PIV). Domestic cats were divided randomly into four treatment groups: saline injection, low and high dose oral, and subcutaneous vaccines. Blood was collected from domestic cats on days 0, 7, 21, 28, and 165 or 208 PIV. Sera were tested for CDV antibodies by virus neutralization. All individuals were seronegative at the beginning of the study. One tiger vaccinated subcutaneously developed a titer of 32 by day 35, which reduced to 16 by day 112. Another tiger vaccinated by tonsillar application developed a titer of 8 on day 112. All other tigers remained seronegative. Cats that received saline injection or oral vaccination remained seronegative at each sampling time. Cats vaccinated subcutaneously developed titers ranging from 4 to > 128 by day 28, and those re-bled beyond day 165 had titers of 16 or 64. The disparity in response between domestic cats and tigers may be due to species differences or a dose-dependent effect. Subcutaneous vaccination with canarypox-vectored PureVax Ferret Distemper is safe and elicits persistent antibody titers in domestic cats vaccinated parenterally.

**Key words:** Canine distemper virus, oral administration, *Panthera tigris*, Purevax Ferret Distemper vaccine®, tiger, vaccination

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## A RETROSPECTIVE REVIEW OF THE CLINICAL VALUE OF FECAL BACTERIAL ENTERIC PATHOGEN CULTURES IN MAMMALS WITHIN A ZOOLOGICAL COLLECTION AND THE USE OF FECAL CYTOLOGY FOR OPTIMIZATION OF ADDITIONAL DIAGNOSTIC TESTING

*Rick Samu, BVM&S, MRCVS,<sup>1\*</sup> Nicole I. Stacy, DVM, DrMedVet, DACVP,<sup>2</sup> and Geoffrey W. Pye, BVSc, MSc, Dipl ACZM<sup>2</sup>*

*<sup>1</sup>From the Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Midlothian EH25 9RG, UK; <sup>2</sup>Disney's Animals, Science, and Environment, 1200 N Savannah Circle, Bay Lake, FL 32830 USA*

### Abstract

The clinical value of fecal bacterial enteric pathogen cultures (FBEP) as part of routine preventive medicine protocols in mammals is unknown. The objectives of this study were to 1) investigate the clinical utility of FBEP results in context of subsequent clinical actions and to 2) present the use of fecal cytology as a tool for optimization of additional diagnostic testing. Retrospective FBEP culture results from 692 tests of 432 mammals taken at Disney's Animal Kingdom from 2011-2016 were separated into preventive (P;  $n = 485$ ), diagnostic (D;  $n = 177$ ), or recheck (R;  $n = 30$ ) samples, and the results and subsequent clinical actions were assigned a clinical significance factor (CSF) of 1 to 5 for D and P samples. 53 FBEP isolated bacterial organisms, with 28 yielding clinically relevant findings. *Aeromonas* spp. was most frequently isolated, followed by *Campylobacter* spp., and *Salmonella* spp. Only 26.4% ( $n = 14$ ; P  $n = 2$ , D  $n = 9$ , R  $n = 3$ ) warranted treatment. A CSF of 3 to 5 was more frequent in D (55%) compared to P (7.4%) samples. Based on these results, using FBEP as part of preventive medicine protocols is not warranted as a routinely included test in mammals. Implementation of fecal cytology as an initial step in fecal evaluation resulted in a prompt and substantial reduction in number of submitted FBEP ( $n = 12$ /mo before and  $n = 5$ /mo after implementation). Fecal cytologic evaluation can provide guidance for clinically useful and cost-effective selection of additional fecal diagnostic testing.

**Key words:** Enteric pathogen screen, fecal culture, fecal cytology, preventive medicine, wellness

### ACKNOWLEDGMENTS

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## A PROPOSED RADIOLOGIC SCORING SYSTEM FOR SHOULDER AND ELBOW OSTEOARTHRITIS IN GERIATRIC NONHUMAN PRIMATES

*Megan E. Freeman, DVM,<sup>1\*</sup> Sophie Dennison, BVM&S, Dipl ACVR,<sup>2</sup> and Cora L. Singleton, DVM, Dipl ACZM<sup>3</sup>*

*<sup>1</sup>Oradell Animal Hospital, Paramus, NJ 07601 USA; <sup>2</sup>TeleVet Imaging Solutions, PLLC, Oakton, VA 22124 USA; <sup>3</sup>San Diego Zoo, San Diego Zoo Global, San Diego, CA 92101 USA*

### Abstract

Shoulder and elbow osteoarthritis (OA) can be a debilitating and painful disease. In humans, pain experience is complex and multifactorial.<sup>4</sup> Nonhuman primates are likely similarly affected by OA but radiologic assessment of OA is subjective and pain determination is challenging.

This study proposes a standardized index for objective radiologic evaluation of osteoarthritic changes of the shoulder joint and elbow joint of nonhuman primates. Ideal joint positioning for different imaging modalities is defined. After review of human OA scoring systems,<sup>1-3</sup> four hallmark radiologic features are selected for evaluation of the shoulder and elbow joints: joint space narrowing, subchondral sclerosis, osteophyte formation, and intra-articular loose body formation. Each joint is scored for each of these four parameters on a five-point scale. Total score for each joint is used to classify the joint as having absent, mild, moderate, severe, or profound osteoarthritis.

A standardized OA scoring system will facilitate thorough evaluation and documentation of joint changes over time. Scores for shoulder and elbow OA can be combined with OA scores for hips, knees, and spine to give an overall assessment of degenerative changes that may cause pain and impact mobility. With heightened awareness of indicators of early OA, targeted medical, behavioral, environmental, and physical interventions may improve animal comfort and welfare and ideally slow the progression of this debilitating disorder.

**Key words:** Elbow, geriatric, nonhuman primate, osteoarthritis, radiologic, shoulder

### ACKNOWLEDGMENTS

The authors thank Dr. Melissa King-Smith and Dr. Emmanuelle Furst for their development of osteoarthritis scoring systems for the hips/knees and spine, respectively.

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## RETROSPECTIVE EVALUATION OF PROGNOSTIC INDICATORS FOR SURVIVAL IN 1256 ORPHANED EASTERN COTTONTAIL RABBITS (*Sylvilagus floridanus*)

Stephanie L. Principati, VMD,<sup>1,2</sup> Krista A. Keller, DVM, Dipl ACZM,<sup>1\*</sup> Matthew C. Allender, DVM, MS, PhD, Dipl ACZM,<sup>1,2</sup> Sarah Reich, DVM,<sup>2,3</sup> and Julia Whittington, DVM<sup>2,3</sup>

<sup>1</sup>Wildlife Epidemiology Laboratory; <sup>2</sup>Department of Veterinary Clinical Medicine; <sup>3</sup>Wildlife Medical Clinic, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 USA

### Abstract

Orphaned eastern cottontail rabbits (ECR), *Sylvilagus floridanus*, are commonly presented to wildlife medical facilities and necessitate large personnel and financial allocations. The purpose of this study was to determine prognostic indicators for survival in orphaned ECR to maximize their survival and ensure appropriate utilization of limited resources at wildlife medical clinics and rehabilitation centers. A total of 1256 ECR presenting to the University of Illinois Wildlife Medical Clinic from 2012-2018 fitting the inclusion criteria were included. Rabbits were identified as survivors (surviving, transferred, or released within 72 hr of presentation) or non-survivors (euthanized or died within 72 hr of presentation). Presenting weight, body system abnormalities, reason for presentation, hydration status, and singleton versus group presentation were categorically recorded for each case. A best fit logistic regression model determined that ECR weighing  $\leq 70$  grams (OR = 2,  $P < 0.0001$ ), presenting as singletons (OR:2,  $P < 0.0001$ ) exhibiting mild integumentary system abnormalities (OR:1.87;  $P = 0.0261$ ) or moderate-severe integumentary abnormalities (OR:4.53,  $P < 0.0001$ ), and those exhibiting neurologic system abnormalities (OR:4.48,  $P < 0.0001$ ) were more likely to have a non-survivor status. Wildlife medical clinics and rehabilitation centers can use the data presented here to guide the triage and therapy and/or euthanasia protocols for orphaned ECR. Future research should be pursued to determine therapeutic methods that can be utilized to maximize survival status in this species.

**Key words:** Eastern cottontail rabbits, lagomorph, prognosis, *Sylvilagus floridanus*, wildlife rehabilitation

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## A PROPOSED RADIOLOGIC SCORING SYSTEM FOR OSTEOARTHRITIS IN THE HIPS AND KNEES OF NONHUMAN PRIMATES

*Melissa King-Smith, DVM,<sup>1\*</sup> Sophie Dennison, BVM&S, Dipl ACVR,<sup>2</sup> and Cora L. Singleton, DVM, Dipl ACZM<sup>3</sup>*

*<sup>1</sup>BluePearl Emergency Veterinary and Specialty Animal Hospital, New York, NY 10019 USA; <sup>2</sup>TeleVet Imaging Solutions, PLLC, Oakton, VA 22124 USA; <sup>3</sup>San Diego Zoo, San Diego Zoo Global, San Diego, CA 92101 USA*

### Abstract

Osteoarthritis (OA) is known to cause chronic debilitating changes to the skeletal system in all types of animals. Known for its ability to incapacitate humans, nonhuman primates maintain activity in the face of severe OA thus challenging assessment of pain. In addition, systematic evaluation of nonhuman primate joints for OA is infrequently applied, and correlates between radiologic changes to the joint and pain in nonhuman primates are lacking.

The aim of this study is to develop a radiologic scoring system for objective assessment of osteoarthritic changes of the hips and knees of nonhuman primates. Ideal joint positioning for different imaging modalities is defined. After review of human OA scoring systems,<sup>1-3</sup> four key radiologic parameters are selected for evaluation of the hip joint and the knee joint: joint space narrowing, subchondral sclerosis, osteophyte formation, and bone end deformity. Each joint is scored for each of these four parameters on a five-point scale. Total score for each joint is used to classify the joint as having absent, mild, moderate, severe, or profound osteoarthritis.

A standardized OA scoring system will facilitate thorough evaluation and documentation of joint changes over time. Scores for hip and knee OA can be combined with OA scores of shoulders, elbows, and spine to give an overall assessment of degenerative changes that may cause pain and impact mobility. With heightened awareness of indicators of early OA, targeted medical, behavioral, environmental, and physical interventions may improve animal comfort and welfare and ideally slow the progression of this debilitating disorder.

**Key words:** Coxofemoral, geriatric, knee, nonhuman primate, osteoarthritis, radiologic

### ACKNOWLEDGMENTS

The authors thank Dr. Emmanuelle Furst and Dr. Megan Freeman for their development of osteoarthritis scoring systems for the spine and shoulders/elbows, respectively.

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## EVALUATION OF AN AGED ANIMAL ASSESSMENT TOOL USED FOR QUALITY-OF-LIFE ASSESSMENT AND END-OF-LIFE PLANNING FOR GERIATRIC ZOO ANIMALS

*Kimberly Vinette Herrin, MS, DVM, MANZCVS,<sup>1\*</sup> Larry Vogelnest, BVSc, MVS, MANZCVS,<sup>1</sup> Frances Hulst, BVSc, MVS,<sup>1</sup> Gabrielle Tobias, BVSc, MAppSc,<sup>1</sup> Michelle Campbell-Ward, BSc, BVSc, MANZCVS, DZooMed,<sup>2</sup> Benn Bryant, BVSc, MVS, MANZCVS,<sup>2</sup> and Benjamin Pitcher, BSc, PhD<sup>1</sup>*

*<sup>1</sup>Taronga Conservation Society Australia, Mosman, NSW, 2088 Australia; <sup>2</sup>Taronga Conservation Society Australia, Dubbo, NSW, 2830, Australia*

### Abstract

Early recognition of age-related changes guides appropriate intervention and directs end-of-life planning to support good welfare in aging zoo animals. In 2013, Taronga Conservation Society Australia implemented an aged animal assessment (AAA) tool to objectively assess quality of life to facilitate welfare-focused end-of-life management. The tool also provides opportunity to address age-related changes through medical treatment, nutrition and husbandry.<sup>1</sup>

AAAs are performed when an animal reaches 80% of the expected longevity for the species.<sup>1</sup> The AAA objectively scores criteria that measure physical and psychologic health (compared with an animal in its prime) and includes behavioral assessment, species specific criteria, curatorial and logistical imperatives. The tool generates a cumulative score that correlates with predetermined numeric ranges that suggest a recommended course of action: treatment, dietary or husbandry changes; end of life planning; or euthanasia.

To date, 137 AAAs have been performed on 90 animals from a variety of taxa. Thirty-three had 1-4 follow-up assessments at 6 mo or 1 yr. Four individuals were euthanized as a direct result of the initial AAA. Another 24 were euthanized based on follow-up AAAs or predetermined decisions to euthanize if conditions deteriorated. Approximately 50% of total cases remained stable while nine cases had improved scores following implementation of individualized care plans and early recognition of disease.

The AAA tool allows objective decision-making regarding quality of life of individual animals. Significant improvement in welfare of aged animals has been attributed to early recognition and intervention of age-related changes or recognition that euthanasia is warranted.

**Key words:** Aged animal assessment, end-of-life planning, quality-of-life assessment, welfare

### LITERATURE CITED

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