CONTINUING EDUCATION

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Howdy!....

…and welcome to the Wild West! Everything is bigger in Texas and the 49th AAZV Annual Conference in Frisco will be no exception. The American Association of Zoo Veterinarians continues to grow as an organization and we expect participation at this year’s conference to be high once again. In addition to our growing membership, we will be meeting concurrently with several of our partners, including those that are joining us on a round-up for the very first time (American Association of Fish Veterinarians), those that have seen a few more miles of dust and cactus (Association of Exotic Mammal Veterinarians), and those that have been on the trail with us for as long as we can remember (Nutrition Advisory Group and Association of Amphibian and Reptile Veterinarians). We are happy to share a campfire with all of you and are just as excited about swapping ghost stories as we are hearing everyone’s formal presentations.

As many of you know, this ain’t my first rodeo. I attended my first AAZV conference twenty-one years ago in the sunny beachside town of Puerto Vallarta, Mexico. There wasn’t a wall separating our organization from others then…. and there isn’t one now. Instead of creating barriers to international zoo medicine, I’m happy to see that we’ve spent the last two decades breaking them down. In addition to meeting concurrently with like-minded folks, we’ve also developed stronger collaborations with the European Association of Zoo and Wildlife Veterinarians, the Latin American Association of Wildlife Veterinarians and other colleagues around the world. We welcome each and every one of you that have travelled to the Dallas area to join us for an exceptional meeting.

There are many hard-working people to thank for putting together another great conference: our Scientific Program Committee, including Susie Bartlett, Allison Tuttle, Kristen Phair, Sam Rivera, and Alicia Hahn; our Executive Director’s Office, including Rob Hilsenroth, Adine Nicholson, and Kathy Nemaric; and our local host, including Chris Bonar and his team at the Dallas Zoo. As well as our co-host, Ashley Barratclough and her team at the Dallas World Aquarium. Thanks are also due to your AAZV Executive Committee and leadership of our AAZV committees who are providing the vision and effort to carry this organization into the future.

I’m fixin’ to have a great time and hope that all y’all are too. In addition, I sincerely hope that everyone who attends will renew their drive to make a meaningful difference for wildlife. If you’re not having fun and aren’t making a difference, then you’re missing the point -- we’re part of one of the greatest professions out there and we have an opportunity to make an impact each and every day.

Have a great conference!

R. Scott Larsen
President, American Association of Zoo Veterinarians
Dear Colleagues and Friends,

On behalf of the Scientific Program Committee, welcome to Dallas for the 49th annual AAZV conference!

The Scientific Program Committee strives to provide you with the very best opportunities for continuing education. We recruit experts from the field to provide high quality workshops to allow you to refine your skills or learn about topics with which you are less familiar. We have worked in conjunction with our wonderful hosts at the Dallas Zoo, Drs. Jan Raines, Chris Bonar, Lynn Kramer, and Maren Connolly, to provide some special opportunities including megavertebrate medicine, avian orthopedics, marine mammal anesthesia, and comparative ophthalmology. We are excited to offer these opportunities and hope you will find them helpful in your practice.

Throughout the week, we are also honored to have invited speakers provide us with several longer talks in their area of expertise. Topics will include emerging diseases in reptiles, invertebrate pharmacology, caring for elephants in Southeast Asia, and how the veterinary feed directive will impact our work as zoo veterinarians. We will finish the week with three masterclasses on Friday. Through these classes we hope to strengthen our knowledge of reptile pathology, understand the underlying principles and applications of acute phase protein testing, and the use of stem cell therapy in equines. We are also pleased to offer continued training in leadership, with a workshop on Friday morning.

The majority of the program, however, relies on the contributions you have made. It is your commitment to sharing the knowledge you have gained through research and through practicing your craft that makes the program so valuable. The oral presentations and posters that you contribute allow us all to benefit and advance the care of our captive zoological animals and wildlife. We thank the American College of Zoological Medicine for certifying the continuing education credit, which includes 23.6 credit hours for the program and 20 credit hours for workshops.

We encourage you to provide the SPC with feedback on the content of the conference. Suggestions of topics to cover, speakers to invite, and thoughts on how to make the conference better are always welcomed. Please remember to fill out the online survey to provide feedback, and we invite you to contact us directly to discuss any suggestions in more depth.

Finally, the Committee would like to thank the members of the AAZV office of the Executive Director, including Rob Hilsenroth, Adine Nicholson, Julie Fazlollah, and Kathy Nemaric for their amazing work helping to organize and execute the conference.

Enjoy the conference, enjoy Dallas, and enjoy spending the week with colleagues and friends!

Best regards,

Susie Bartlett, DVM, Dipl. ACZM
Chair, AAZV Scientific Program Committee
Associate Veterinarian, Wildlife Conservation Society
## American Association of Zoo Veterinarians
### 2017 Scientific Program Committee

<table>
<thead>
<tr>
<th>Position</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCIENTIFIC PROGRAM COMMITTEE CHAIR</td>
<td>Susie Bartlett</td>
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<td>SCIENTIFIC PROGRAM COMMITTEE VICE-CHAIR</td>
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<td>Alicia Hahn</td>
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<td>2017 LOCAL HOST</td>
<td><em>Dallas Zoo</em></td>
</tr>
<tr>
<td></td>
<td>Chris Bonar</td>
</tr>
</tbody>
</table>
CONTENTS

PROGRAM CHAIR:  Kristen Phair

ANESTHESIA
Julie Swenson and Lauren Howard, Chairpersons

WHAT YOU SEE IS NOT WHAT YOU GET: ACCURACY OF NONINVASIVE ANESTHETIC MONITORING IN THE ANESTHETIZED GIRAFFE (Giraffa camelopardalis)

INTRAVENOUS ANESTHESIA PROTOCOLS IN GREAT APES
Shannon T. Ferrell,* Nancy P. Lung, Eric J. Darrow, and Annajane Marlar ............................................. 2

COMPARISON OF A GUAIFENESIN, KETAMINE, AND MEDETOMIDINE CONSTANT-RATE INFUSION WITH ISOFURANE GAS FOR ANESTHESIA MAINTENANCE IN AMERICAN BLACK BEARS (Ursus americanus)

MICROBIAL INTEGRITY STUDY OF PRESERVATIVE-FREE ALFAXALONE (ALFAXAN) IN A MULTI-USE SYSTEM WITH TWO STORAGE CONDITIONS AND THREE HANDLING TECHNIQUES
Michelle C. Whitehead, * Gigi Davidson, Chelsey Vanetten, Megan Jacob, and Tara M. Harrison........... 4

PHARMACOKINETICS AND PHARMACODYNAMICS OF INTRAMUSCULAR ADMINISTRATION OF ALFAXALONE IN PEAFOWL (Pavo cristatus)
Amanda C. Morphet,* Kayla Hasse, Daniel L. Gustafson, Eric Klaphake, and Matthew Johnston .......... 6
COMPARISON OF THE EFFICACY AND SAFETY OF MEDETOMIDINE-KETAMINE TO A NOVEL MEDETOMIDINE-AZAPERONE-ALFAXALONE COMBINATION IN WILD ROCKY MOUNTAIN BIGHORN SHEEP (Ovis canadensis)
Maggie Grover,* Nigel Caulkett, Peter Neuhaus, Kathreen Ruckstuhl, Søren Boysen, and Åsa Fahlman ................................................................. 8

EFFECTS OF COMBINING NITROPRUSSIDE WITH MEDETOMIDINE-AZAPERONE-ALFAXALONE IN CAPTIVE WHITE-TAILED DEER (Odocoileus virginianus)
Kelsey Chapman,* Nigel Caulkett, Søren Boysen, Rob Stevens, and Murray Woodbury ......................... 10

INVESTIGATIONS INTO THE USE OF PLETHYSMOGRAPHIC VARIABILITY INDEX (PVI) IN ANESTHETIZED CAPTIVE TIGERS (Panthera tigris)
Andrew Cushing,* Chris Smith, Edward Ramsay, Xiaojuan Zhu, and Reza Seddighi ......................... 11

AVIAN MEDICINE
J. Jill Heatley and Adrian Mutlow, Chairpersons

INVITED PRESENTATION: AVIAN INFECTIOUS DISEASES
J. Jill Heatley* and Ian Tizard................................................................................................................. 12

APPENDICULAR FRACTURES IN BIRDS OF PREY: A RETROSPECTIVE STUDY, 2000-2015
Thomas Coutant,* Claire Vergneau-Grosset, and Guy Fitzgerald ........................................................... 17

DIAGNOSTIC VALUE OF PLASMA BIOCHEMISTRY, HEMATOLOGY, RADIOGRAPHY, AND ENDOSCOPIC VISUALIZATION IN COMPANION PSITTACINE BIRDS THAT UNDERWENT ENDOSCOPIC LIVER BIOPSY: 28 CASES, 2007-2016
Cher Hung,* Izidora Sladakovic, and Stephen J. Divers......................................................................... 18

HEMOPARASITE INFECTIONS IN SNOWY OWLS (Bubo scandiacus)
Christy L. Rettenmund,* Kendra C. Baker, Samantha J. Sander, Anne E. Rivas, Kaitlin C. Green, Lisa Mangus, and Ellen Bronson ......................................................... 19

PATHOGEN PREVALENCE IN THE YELLOW-EYED PENGUIN (Megadyptes antipodes)
Emily Kay,* Brett Gartrell, Wendi Roe, and Laryssa Howe ................................................................. 21

REVIEW OF MORTALITY AND EFFECTIVENESS OF NEONATAL TREATMENT IN CAPTIVE ATTWATER’S PRAIRIE CHICKENS (Tympanuchus cupido attwateri)
Lauren Mulreany,* Joseph Flanagan, Christine Molter, Lauren Howard, Maryanne Tocidlowski, Stephen Werre, Stanley Vanhooser, and Michael Morrow ......................................................... 22

PHARMACOKINETICS OF A CONCENTRATED BUPRENORPHINE FORMULATION IN RED-TAILED HAWKS (Buteo jamaicensis)
Molly D. Gleeson,* David Sanchez-Migallon Guzman, Heather K. Knych, Philip H. Kass, Tracy L. Drazenovich, and Michelle G. Hawkins ........................................................................... 24

MINIMIZING RISK BY MAXIMIZING INTERVENTION TO PREVENT Salmonella IN AVIARY RAINBOW LORIKEETS (Trichoglossus haematodus)
Meredith M. Clancy* and Nadine Lamberski .......................................................................................... 26
STANDING COMPUTED TOMOGRAPHY IN NONANESTHETIZED LITTLE PENGUINS (Eudyptula minor) TO ASSESS NORMAL ANATOMY AND MONITOR DISEASE
Anne E. Rivas,* Anthony Fischetti, Alexandre B. Le Roux, Charlotte Hollinger, David A. Oehler, Robert P. Moore, and Jean A. Paré ................................................................. 27

EFFECTS OF MIDAZOLAM AND MIDAZOLAM-BUTORPHANOL SEDATION ON GASTROINTESTINAL TRANSIT TIMES IN COCKATIELS (Nymphicus hollandicus)
Anna Martel-Arquette, Grayson Doss, and Christoph Mans* ............................................................... 29

INVERTEBRATES
Trevor Zachariah and Carlos Sanchez, Chairpersons

INVITED PRESENTATION: INVERTEBRATE PHARMACOLOGY
Gregory A. Lewbart ................................................................................................................................. 30

HEMOLYMPH CYTOLOGY, CELL COUNT, AND ELECTROLYTE REFERENCE VALUES IN CAMEROON RED TARANTULA (Hysterocrates gigas)
Cédric B. Larouche,* Janet Beeler-Marfisi, Lydia Attard, Nicole Nemeth, and Hugues Beafrère .............. 40

SWEATING THE SMALL STUFF: APPLICATION OF SCIENTIFIC MANAGEMENT INCREASES LABORATORY REPRODUCTION IN STAGHORN CORAL (Acropora cervicornis)
Linda M. Penfold,* Scott Graves, Cayman Adams, Eneour Puill-Stephan, Kathy Heym, and Margo McKnight ............................................................................................................. 42

LACK OF EFFECT OF INJECTABLE ALFAXALONE IN THE MADAGASCAR HISSING COCKROACH (Gromphadorhina portentosa) AND ORANGE-SPOTTED COCKROACH (Blaptica dubia): A PILOT STUDY
Kevin Sio,* Jon Romano, and Matthew S. Johnston .................................................................................. 43

PRIMATES
Nancy Lung and Karen Kearns, Chairpersons

TREATMENT OF GLAUCOMA IN A MALE GORILLA (Gorilla gorilla gorilla) USING TRANSSCLERAL MICROPULSE LASER THERAPY
John M. Sykes IV,* Kate A. Gustavsen, and John Sapienza .................................................................... 48

CONGENITAL HYPOTHYROIDISM IN A BORNEAN ORANGUTAN (Pongo pygmaeus) AND A SUMATRAIN ORANGUTAN (Pongo abelii)
Melissa A. Fayette,* Maryanne E. Tocidlowski, Tamara N. Kruse, Jeffry S. Proudfoot, and Michelle R. Bowman .................................................................................................................. 49

MANAGEMENT OF Clostridium difficile INFECTION IN A COLONY OF BLACK-HANDED SPIDER MONKEYS (Ateles geoffroyi)
Ava M. Trent,* Matthew J. Hamilton, Alexander Khoruts, Michael J. Sadowsky, Arno Wünschmann, Tami L. Murphy, and Madison L. Johnson .................................................................................................................. 51

REVIEW OF REPRODUCTION IN CAPTIVE WESTERN GORILLAS (Gorilla gorilla), 1996-2016
Erin Berlin,* Mary Thurber, Roby Elsner, and Nadine Lamberski ............................................................... 52
CALLITRICHID PREVENTIVE MEDICINE PROTOCOLS AT THE BRONX ZOO: A CASE EXAMPLE OF THE RE-EVAUATION PROCESS
Matthew Golembeski,* Kenneth J. Conley, and John M. Sykes IV ................................................................. 54

AVOCADO (Persea americana) TOXICITY IN CAPTIVE AYE-AYE (Daubentonia madagascariensis)
Robert L. Schopler,* Cathy V. Williams, and Jeffrey I. Everitt ................................................................. 55

AQUATIC ANIMAL MEDICINE
Sara Childs-Sanford and Kirsten Gilardi, Chairpersons

APPLICATION OF COMMERCIAL Aspergillus WESTERN BLOT IGG® KIT AND ELISA ASSAY FOR THE DIAGNOSIS OF ASPERGILLOSIS IN COMMON BOTTLENOSE DOLPHINS (Tursiops truncatus)
Guillaume Desoubeaux, Carolina Le-Bert, Vanessa Fravel, Tonya Clauss, Alexa J. Delaune, Jeny Soto, Risa Daniels, Eric D. Jensen, Celeste Parry, Jennifer E. Flower, Randall Wells, Gregory D. Bossart, and Carolyn Cray* ............................................................................................................................................ 57

RETROSPECTIVE REVIEW OF MORBIDITY AND MORTALITY IN FROGFISH (ANTENNARIIDAE), 2002-2015
Kanyon M. McLean,* Stephanie T. Munyon, Karen A. Terio, William Van Bonn, Caryn P. Poll, and Matthew O’Connor ..................................................................................................................................... 59

COMPARISON OF A SMARTPHONE-BASED ELECTROCARDIOGRAM DEVICE WITH A STANDARD SIX-LEAD ELECTROCARDIOGRAM IN THE ATLANTIC BOTTLENOSE DOLPHIN (Tursiops truncatus)
Taylor J. Yaw,* Marc S. Kraus, Allison C. Ginsburg, Leigh A. Clayton, Catherine A. Hadfield, and Anna R. Gelzer ..................................................................................................................................................... 60

DETECTION OF HERPESVIRUS IN WILD POPULATIONS OF SOUTH AMERICAN SEA LIONS (Otaria byronia) AND PERUVIAN FUR SEALS (Arctocephalus australis) IN PERU
Karisa Tang,* Michael J. Adkesson, Laura Adamovicz, Galaxia Córtes-Hinojosa, Susana Cárdenas-Alayza, and Matthew C. Allender ......................................................................................................... 62

RETROSPECTIVE ANALYSIS OF PERIANESTHETIC MORTALITY RISK FACTORS IN CALIFORNIA SEA LIONS (Zalophus californianus) UNDERGOING REHABILITATION
Justin F. Rosenberg,* Shawn P. Johnson, F. Fabian Okonski, Sophie Whoriskey, Claire A. Simeone, and Cara L. Field ........................................................................................................................................... 64

EVALUATION OF SERUM PROLACTIN AS A MEANS OF PREGNANCY DIAGNOSIS IN FLORIDA MANATEES (Trichechus manatus latirostris)
Melissa R. Nau,* Lauren N. Smith, Donald L. Thompson, Jr., and Ray Ball .......................................................................................................................... 66

INFLUENCE OF IODINE SUPPLEMENTATION ON SERUM T<sub>3</sub> AND T<sub>4</sub> CONCENTRATIONS IN WHITE-SPOTTED BAMBOO SHARKS (Chiloscyllium plagiosum)
Lily A. Parkinson,* Terry W. Campbell, and Kurt Sladky .................................................................................. 67

SIGNIFICANCE OF SYMMETRIC DIMETHYLARGININE (SDMA) IN EVALUATING RENAL INSUFFICIENCY IN REHABILITATED WILD FLORIDA MANATEES (Trichechus manatus latirostris) AND REFERENCE VALUES IN TWO WILD MANATEE POPULATIONS
Lauren N. Smith,* Robert K. Bonde, Melissa R. Nau, and Ray L. Ball ........................................................................ 68
## REPTILES AND AMPHIBIANS
*Matt Allender and Joe Flanagan, Chairpersons*

### INVITED PRESENTATION: EMERGING DISEASES IN REPTILES AND HOW WE MAY BE CHANGING OUR DEFINITION OF HEALTH
*Matt Allender* ................................................................................................................................. 70

### NOVEL Parananniziopsis SPECIES IN A WAGLER’S VIPER (*Tropidolaemus wagleri*) AND TENTACLED SNAKE (*Erpeton tentaculatum*) IN A ZOOLOGICAL COLLECTION
*Kimberly L. Rainwater,* Nathan P. Wiederhold, Deanna A. Sutton, Michael M. Garner, Cheryl Maguire, Carmita Sanders, Connie Gibas, José F. Cano, Josep Guarro, and Alberto M. Stchigel* .......................................................................................................................... 71

### DIAGNOSTIC PERFORMANCE OF READILY AVAILABLE ANALYTES IN THE DIAGNOSIS OF INFLAMMATION IN GOPHER TORTOISES (*Gopherus polyphemus*)
*Justin F. Rosenberg,* James F. X. Wellehan, Jr., Jorge A. Hernandez, Sarah E. Crevasse, Carolyn Cray, and Nicole I. Stacy ........................................................................................................................................ 72

### TREATMENT OF CHYTRIDIOMYCOSIS WITH F10 VETERINARY DISINFECTANT
*Ché Weldon* Marizaan de Jong, and Ryno van Dyk ........................................................................... 74

### RETROSPECTIVE EVALUATION OF MYCOBACTERIOSIS IN THE HOUSTON TOAD (*Anaxyrus houstonensis*): CLINICAL PRESENTATIONS, PATHOLOGIC FINDINGS, AND PREVENTION
*Gregory Walth,* Eric Snook, and Lauren Howard ..................................................................................... 75

### WHIP-LIKE HETEROPHIL PROJECTIONS IN REPTILES: ART, ARTIFACT, OR SUPPORTIVE OF INFLAMMATION?
*Nicole I. Stacy,* Terry M. Norton, Daniel V. Fredholm, Carlos Rodriguez, Lidia Castro, Craig Pelton, and John W. Harvey ........................................................................................................................................ 76

### PERIPHERAL NERVE SHEATH TUMORS IN A CAPTIVE ASSURANCE COLONY OF HOUSTON TOADS (*Anaxyrus houstonensis*)
*Lauren L. Howard,* Eric Snook, and Anibal G. Armién ............................................................................ 78

### HYPERVISCOSITY-LIKE SYNDROME IN REPTILES
*Elise E. B. LaDouceur* and Michael M. Garner ..................................................................................... 79

## CARNIVORES
*Erika Crook and Gwen Myers, Chairpersons*

### ADDRESSING THE CHALLENGE OF DO-IT-YOURSELF (DIY) SEMEN BANKING IN WILD FELIDS
*William F. Swanson,* Lindsey M. Vansandt, Scott Citino, R. Scott Larsen, Gretchen A. Cole, and Anneke Moresco ........................................................................................................................................ 81

### CAPSULE ENDOSCOPY AS A NOVEL TOOL FOR GASTROINTESTINAL DISEASE DIAGNOSIS IN A PUMA (*Felis concolor*)
*Jessica N. Lovstad,* Jill Pomrantz, and Kathryn C. Gamble ....................................................................... 83
COCCIDIOSIS IN THE ENDANGERED BLACK-FOOTED FERRET (*Mustela nigripes*): NEW INVESTIGATIONS INTO AN OLD DISEASE
Adriana R. Pastor,* John R. Barta, Simon Hollamby, and Dale A. Smith................................. 85

SURGICAL LIP-TO-LID TRANSPOSITION CORRECTION AND HUSBANDRY MANAGEMENT FOR SEVERE EYELID COLOBOMA IN A LITTER OF SNOW LEOPARDS (*Uncia uncia*)
Jen J. Kilburn,* Kay A. Backues, and Jonathan Pucket............................................................... 86

RETROSPECTIVE ANALYSIS OF SERUM SYMMETRIC DIMETHYLARGININE (SDMA) CONCENTRATIONS IN CHEETAHS (*Acinonyx jubatus*) WITH KIDNEY DISEASE
Lynnette Waugh,* Shane Lyon, Gretchen A. Cole, Jennifer D’Agostino, Julie Cross, Marilyn Strong-Townsend, Maha Yerramilli, Jun Li, Andrei Rakitin, Sean Hardy, and João Brandão.......................... 87

PRELIMINARY POPULATION PHARMACOKINETICS OF MELOXICAM IN LION (*Panthera leo*), CHEETAH (*Acinonyx jubatus*), AND TIGER (*Panthera tigris*)
Marike Visser,* Ellen Bronson, and Dawn Boothe ....................................................................... 89

INVITED PRESENTATION: MEDICALLY IMPORTANT ANTIMICROBIALS IN FOOD-PRODUCING ANIMALS AFTER 1 JANUARY 2017
Mike Murphy...................................................................................................................................... 91

EMERGING INFECTIOUS DISEASES
Maren Connolly and John Sykes, IV, Chairpersons

FATAL RANAVIRUS OUTBREAK IN A CAPTIVE GROUP OF MELLER’S CHAMELEONS (*Trioceros melleri*)
Samantha J. Sander,* Lauren Peiffer, Kathleen Gabrielson, Allan Pessier, Matthew C. Allender, Thomas Waltzek, and Ellen Bronson ................................................................. 92

CANINE DISTEMPER VIRUS OUTBREAK IN CAPTIVE LINNAEUS’S TWO-TOED SLOTHS (*Choloepus didactylus*): CLINICAL AND PATHOLOGIC FINDINGS AND RESPONSE TO VACCINATION
Julie D. Sheldon,* Andrew C. Cushing, Rebecca P. Wilkes, Eman Amis, and Edward J. Dubovi ................................................................................................................................. 94

CLINICAL COWPOX INFECTION IN TWO GIANT ANTEATERS (*Myrmecophaga tridactyla*)
Ian Ashpole,* Steve Unwin, and Julian Chantrey ............................................................................. 96

GASTROINTESTINAL DISEASE ASSOCIATED WITH NON-ALBICANS Candida SPECIES IN BIRDS
Kyle Donnelly,* James F. X. Wellehan, Jr., and Katherine Quesenberry ........................................... 97

TOOLS FOR THE EXOTIC ANIMAL INDUSTRY FROM THE SECURE ZOO STRATEGY PROGRAM
Yvonne Nadler,* Jimmy Tickel, Jeanie Lin, and Steve Olson ........................................................... 98
ELEPHANTS AND RHINOCEROS
Tracy Clippinger and Jennifer D’Agostino, Chairpersons

INVITED PRESENTATION: SUPPORTING ALTERNATIVES TO TRADITIONAL ELEPHANT TRAINING METHODS IN SOUTHEAST ASIA
Gerardo Martinez ........................................................................................................................................ 99

USE OF INTERLEUKIN RECEPTOR ANTAGONIST PROTEIN (IRAP) IN A MULTI-MODAL THERAPEUTIC REGIME FOR OSTEOARTHRITIS IN AN ASIAN ELEPHANT (Elephas maximus)
Jessica L. Siegal-Willott,* Paul Anikis, Donald L. Neiffer, Tony Barthel, and Laurie Goodrich................................................................. 100

TUBERCULOSIS CAUSED BY Mycobacterium orygis IN A GREATER ONE-HORNED RHINOCEROS (Rhinoceros unicornis): FIRST REPORT IN THE WESTERN HEMISPHERE
David Love,* Michael M. Garner, Konstantin P. Lyashchenko, Alina Sikar-Gang, Michele Miller, Daniel S. Bradway, Suelee Robbe-Austerman, and Jan Ramer .................................................. 102

SUDDEN DEATH IN THREE SOUTHERN WHITE RHINOCEROS (Ceratotherium simum simum) SECONDARY TO PRESumptive Clostridium perfringens ENTEROTOXEMIA
Scott Citino,* Alexandra Goe, Lara Metrione, Marcie Oliva, and Michael Garner ............................................. 105

NEGATIVE EFFECTS OF ANALGESIC AND ANESTHETIC DRUGS ON SPERM MOTILITY: IMPLICATIONS FOR ASSISTED BREEDING IN MANAGED RHINOCEROS
Jack Kottwitz,* Monica Stoops, Jaida Reeves, Roy Harmon, Robyn Wilborn, Misty Edmondson, and Dawn Boothe ................................................................. 107

CHARACTERIZATION AND ADMINISTRATION OF ALLOGENEIC BLOOD-DERIVED MESENCHYMAL STEM CELLS IN AN AFRICAN ELEPHANT (Loxodonta africana) WITH SEVERE OSTEOARTHRITIS
Valerie Johnson,* Matthew Johnston, Liza Dadone, and Steven Dow ................................................................. 109

IN-DEPTH ANALYSIS OF THE VITAMIN D AND CALCIUM STATUS OF ASIAN ELEPHANTS (Elephas maximus) MANAGED IN A NORTHERN TEMPERATE CLIMATE
Sara E. Childs-Sanford,* Andrew J. Makowski, and Joseph J. Wakshlag ................................................................. 111

WILDLIFE
Sonia Hernandez and Karen Wolf, Chairpersons

VISCERAL LEPIDOPTERISM IN CAPTIVE OTARIIDS DUE TO MIGRATION OF CATERPILLAR Setae ASSOCIATED WITH SYSTEMIC VASCULOPATHY AND DEATH
Lydia J. Tong,* Frances Hulst, Gabrielle Tobias, Kimberly Vinette Herrin, Phoebe Meagher, and Larry Vogelnest ................................................................. 113

ARE SALAMANDERS SAFE? HEALTH ASSESSMENT OF THE SILVERY SALAMANDER (Ambystoma platinuem) IN VERMILION COUNTY, ILLINOIS USA PRIOR TO AND DURING A RANAVIRUS MORTALITY EVENT
Laura Adamovicz,* Chris Phillips, Kelsey Low, Kayla Boers, and Matthew C. Allender ................................................................. 115
SARCOCYSTOSIS IN A FLOCK OF THICK-BILLED PARROTS (Rhynchopsitta pachyrhyncha), 2005-2016: MORBIDITY, MORTALITY, DIAGNOSTICS, AND MANAGEMENT STRATEGIES
Anne E. Rivas,* Kenneth Conley, Tracie A. Seimon, Charlotte Hollinger, Robert P. Moore, and Jean A. Paré .......................................................... 117

EVALUATION OF A NOVEL HERPESVIRUS AS A SENTINEL FOR POPULATION HEALTH IN ENDANGERED BLANDING’S TURTLES (Emydoidea blandingii)
Dana M. Lindemann,* Matthew C. Allender, Dan Thompson, Gary A. Glowacki, Erin Newman, and Laura A. Adamovicz .............................................. 118

DRIVERS AND IMPACTS OF SINGLE AND CO-PATHOGEN OCCURRENCE IN FREE-LIVING EASTERN BOX TURTLES (Terrapene carolina carolina) IN ILLINOIS AND TENNESSEE USA, 2013-2016
Laura Adamovicz* and Matthew C. Allender ........................................................... 120

HOOFSTOCK
Holly Haefele and Danelle Okeson, Chairpersons

RETROSPECTIVE CHARACTERIZATION OF REPRODUCTIVE TRACT LESIONS IN RELATION TO AGE, PARITY, AND CONTRACEPTION IN CAPTIVE FEMALE SUIDAE AND TAYASSUIDAE
Camille C. Goblet* Anneke Moresco, Dalen Agnew, Michael Garner, and Annie E. Newell-Fugate ........................................................................ 122

AMYLOID IN CAPTIVE BONGO (Tragelaphus eurycerus): IMPACTS ON MORBIDITY AND MORTALITY AND EVALUATION OF SERUM ACUTE PHASE PROTEINS PREMORTEM
Susan L. Bartlett,* Carolyn Cray, Nadine Lamberski, and Michael M. Garner .......................................................... 124

SUSPECTED MOXIDECTIN TOXICOSIS IN THREE SPECIES OF HOOFSTOCK AT A SEMI-FREE RANGE ZOOLOGICAL PARK
Julie Swenson* and Holly J Haefele ........................................................................ 125

ALPHAHERPESVIRUS OUTBREAK ASSOCIATED WITH MORTALITY IN A GROUP OF GREVY’S ZEBRA (Equus grevyi) HOUSED IN A MIXED-SPECIES EXHIBIT: DIAGNOSIS, MANAGEMENT AND SURVEILLANCE
Antoine Leclerc,* Loïc Legrand, Nicolas Goddard, Amélie Nicolau, Stéphane Pronost, and Baptiste Mulot ........................................................................ 126

SILICONE MATRIX EPISCLERAL CYCLOSPORINE IMPLANT FOR TREATMENT OF CORNEAL DISEASE IN MALAYAN TAPIR (Tapirus indicus)
Marisa Bezjian,* Lorraine Karpinski, Gabriella Flacke, and Gwen Myers ...................... 128

SYSTEMIC AMYLOIDOSIS IN A POPULATION OF PRONGHORN ANTELOPE (Antilocapra americana)
Margaret E. Martinez, Dawn M Zimmerman,* Katie Seeley, Priya Bapodra, Liwen Zhang, and Rachel Cianciolo ............................................................... 129

EFFECTS OF A HIGH-PHYTOESTROGEN DIET ON EQUINE ESTROUS CYCLES AND FERTILITY USING DOMESTIC MARES (Equus caballus) AS MODELS FOR WILD UNGULATES
Mollie Samocha,* Troy Tollefson, Nicole Sharpe, and Bruce W. Christensen ................. 130
HUMAN MEDICAL EXPERIENCE PROVIDES PARADIGMS RELEVANT TO CAPTIVE BREEDING OF ENDANGERED WILDLIFE: RATIONALE FOR PREVENTION AND THERAPY OF HEMOLYTIC AND IRON OVERLOAD PROPENSITIES IN BROWSER RHINOCEROSES, TAPIRS, AND OTHER SUSCEPTIBLE SPECIES
Donald E. Paglia ................................................................. 132

BEHAVIOR, WELFARE, AND LEADERSHIP
Mads Bertelsen, Clay Hilton, and Marion Desmarchelier, Chairpersons

A MULTIFACETED PROGRAM FOR END-OF-LIFE CARE
Elizabeth C. Nolan* and David A. Orban ................................................................. 142

ADDITION OF COMPLEMENTARY THERAPIES TO ZOOLOGIC MEDICINE
Jessica A. Marziani,* Christine M. Molter, and Lauren L. Howard ........................................ 143

CAREGIVER PLACEBO EFFECT ON ZOO-ANIMAL WELFARE: APPLICATION OF A PRESSURE WALKWAY SYSTEM FOR OBJECTIVE EVALUATION OF LAMENESS IN A ZOO SETTING
Michael J. Adkesson,* Julie Balko, and Sathya K. Chinnadurai .............................................. 146

ALLOSTATIC LOAD: QUANTIFYING CHRONIC AND LONG-TERM INTERMITTENT STRESS TO IMPROVE HEALTH AND WELL-BEING IN ANIMALS
Barbara Wolfe,* Ashley Edes, Kathryn Seeley, Kathryn Proudfoot, Elizabeth Berkeley, and Douglas E. Crews .......................................................... 148

ENHANCING CONSERVATION THROUGH VETERINARY CARE OF THE WHITE-BELLIED TREE PANGOLIN (Manis tricuspis)
Copper Aitken-Palmer,* Ginger L. Sturgeon, Jonathan Bergmann, Felicia Knightly, James G. Johnson III, Marina Ivančić, Thomas W. deMaar, Deborah A. Carboni, and Michael Adkesson .................................................. 150

ORGANIZATIONAL INFLUENCE: WHAT ZOO LEADERS SAY ABOUT VETERINARIANS
Donald L. Janssen .............................................................................................................. 151

DIRECT INSPIRATION: ZOO VETERINARY COMMUNITY OUTREACH OPPORTUNITIES
John M. Sykes IV ............................................................................................................. 154

TIPS, TRICKS, AND CASE REPORTS
Maryanne Tocidlowski and Benji Alcantar, Chairpersons

DENTAL DISEASE AND A LEFT DISPLACEMENT OF THE ABOMASUM IN A RETICULATED GIRAFFE (Giraffa camelopardalis reticulate): LESSONS LEARNED
Kimberly A. Thompson,* Ronan Eustace, Colleen Monahan, Colleen Turner, and Vengai Mavangira ................................................................................................................. 155

GENERAL ANESTHESIA AND SURGERY TO TREAT A MANDIBULAR SEQUESTRUM IN AN ATLANTIC BOTTLENOSE DOLPHIN (Tursiops truncatus)
Lara A. Croft,* Stacy DiRocco, Michelle Davis, Todd Schmitt, James Bailey, and Dean Hendrickson .................................................................................................................... 157
LONG-TERM MANAGEMENT OF FUNGAL PNEUMONIA (*Coccidioides immitis*) IN A KIRK’S DIK-DIK (*Madoqua kirkii*) IN A HIGHLY ENDEMIC AREA
*Kari E. Musgrave* Kristen Phair, and Gary West .................................................................................. 158

CARDIAC PACEMAKER IMPLANTATION FOR MANAGEMENT OF ATRIOVENTRICULAR BLOCK IN TWO TASMANIAN DEVILS (*Sarcophilus harrisii*)
*Dennis Michels,*, *Cora Singleton, Sarah E. Achen, Joao Orvalho, and Ric Berlinski* .................................. 159

CONGENITAL DILATED CARDIOMYOPATHY WITH CONGESTIVE HEART FAILURE IN A RHINOCEROS HORNBILL (*Buceros rhinoceros*)
*Kari E. Musgrave*, *Whit M. Church, Kristen Phair, and Gary West* ............................................................... 160

A PRIMARY URINARY BLADDER TERATOMA IN A MANED WOLF (*Chrysocyon brachyurus*)
*Lana Fox*, *Christopher S. Hanley, Luis R. Padilla, and Mary Duncan* ............................................................ 161

DIAGNOSIS AND TREATMENT OF HYPERTHYROIDISM IN A GUANACO (*Lama guanicoe*)
*Christina E. McCullough*, *Matt D. Miesner, and Jessie D. Monday* .............................................................. 162

EVALUATION OF AMANTADINE IN A MULTIMODAL ANALGESIC REGIMEN FOR ALLEVIATION OF OSTEOARTHRITIS IN MULTIPLE CAPTIVE ZOO SPECIES
*Tamara N. Kruse*, *Michelle R. Bowman, Melissa A. Fayette, and Jeffry S. Proudfoot* .................................. 164

MASTERCLASSES
*Kristen Phair*, Chairperson

GROSS LESION RECOGNITION IN ZOO REPTILES
*Michael M. Garner* ........................................................................................................................................ 165

REGENERATIVE MEDICINE IN LAMENESS AND ORTHOPEDICS
*Ashlee E. Watts* .............................................................................................................................................. 166

HOW, WHAT, AND WHY OF TRACKING THE ACUTE PHASE RESPONSE
*Carolyn Cray* ............................................................................................................................................ 172

POSTERS
*Meredith Clancy, Adrienne Atkins, James Steeil, Debbie Myers and Sam Sander*, Chairpersons

REPTILES, AMPHIBIANS, AND INVERTEBRATES

MODIFIED CHOUKRON’S PLATELET-RICH FIBRIN USED AS A TOPICAL WOUND TREATMENT IN A CAPTIVE ELONGATED TORTOISE (*Indotestudo elongata*)
*Ting-Yu Chen* ................................................................................................................................................ 178

ATLAS OF INVERTEBRATE FEED SPECIES: DISTINGUISHING FRIEND FROM FOE
*Eileen Henderson* and *Dalen Agnew* ........................................................................................................ 179

EVALUATION OF THE USE OF KETAMINE-DIAZEPAM COMBINATIONS FOR IMMOBILIZATION OF AFRICAN LAND TORTOISE (*Geochelone spp.*)
*Veronica E. Adetunji*, *John O. Ogunsola, and Olanike K. Adeyemo* ......................................................... 180
TO STAB OR TO SHOWER? ALFAXALONE ANESTHESIA IN COLORADO RIVER TOADS (Incilius alvarius) BY INTRAMUSCULAR INJECTION OR TOPICAL APPLICATION
Stamatios A. Tahas,* Sandra Wenger, Tina M. Binz, and Jean-Michel Hatt ...................................................... 181

PHARMACOKINETICS OF SUBCUTANEOUS HYDROMORPHONE ADMINISTRATION IN BEARDED DRAGONS (Pogona vitticeps)
Shawna Cikanek,* Sherry Cox, Taylor J. Yaw, and Kurt Sladky................................................................. 182

ARE CROSS-SECTIONAL HEALTH DATA REALLY GIVING US ENOUGH INFORMATION TO CHARACTERIZE POPULATIONS? ENHANCING CHELONIAN HEALTH THROUGH A PROSPECTIVE COHORT STUDY
Jeremy M. Rayl,* Matthew C. Allender, Laura Adamovicz, and Marta Rzadkowska .............................. 183

COMPARISON OF ALFAXALONE, TILETAMINE/ZOLAZAPAM, AND DEXMEDETOMIDINE INJECTABLE PRE-ANESTHETICS AND IMMERSION IN TRICAINE METHANESULFONATE (MS-222) FOR SURGICAL LEVEL ANESTHESIA IN THE HOUSTON TOAD (Anaxyrus houstonensis)
Maryanne E. Tocidlowski .............................................................................................................................. 184

LIMITATIONS AND IMPACTS OF THE RENAL PORTAL SYSTEM ON THE DETERMINATION OF THE GLOMERULAR FILTRATION RATE USING CONTRAST-ENHANCED COMPUTED TOMOGRAPHY AND PLASMA CLEARANCE OF IOHEXOL IN BEARDED DRAGONS (Pogona vitticeps)
Marjorie Bercier,* Robson F. Giglio, Matthew D. Winter, and James F. X. Wellehan, Jr ...................... 185

BEHAVIOR AND WELFARE

CYCLOBENZAPRINE AS A POTENTIAL MEANS OF PHARMACOLOGIC MANAGEMENT OF STEREOTYPIC BEHAVIORS IN BEARS
McKenzie A. George,* Jack Kottwitz, and Dawn Boothe................................................................. 187

Kanyon M. McLean* and Geoffrey W. Pye .............................................................................................. 189

INCIDENCE OF CLAW DISORDERS IN GERIATRIC CAPTIVE SLOTH BEARS (Melursus ursinus) AND ITS MANAGEMENT
Rashmi S. Gokhale,* Arun A. Sha, S. Ilayaraja, Pushpendra Kumar Singh, and M. V. Sharma ................................................................. 190

CAPTIVE BEAR WELFARE
Laurie J. Gage,* Andrea D’Ambrosio, Tonya Hadjis, Carolyn McKinnie, and Nicolette Petervary ................................................................. 192

FREEDOMS, PROVISIONS, AND AIMS: IMPROVING CONCEPTUAL FRAMEWORKS FOR ZOO-ANIMAL WELFARE
Monica List* and Sally A. Nofs.................................................................................................................. 195
WILDLIFE

BIOMEDICAL EVALUATION OF SAHAMALAZA SPORTIVE LEMURS (*Lepilemur sahamalaza*) IN THE NATIONAL PARK OF SAHAMALAZA-ILES RADAMA, MADAGASCAR
Michelle Barrows, Rowena Killick,* and Charlotte Day .............................................................. 196

RETROSPECTIVE HISTOPATHOLOGIC FINDINGS IN FREE-RANGING CALIFORNIA HUMMINGBIRDS
Michelle Magagna,* Erica Noland, Lisa Tell, Guthrum Purdin, and Dalen Agnew ....................... 197

AQUATIC ANIMALS

EVALUATION OF A SEMI-QUANTITATIVE LATERAL FLOW DEVICE FOR SERUM AMYLOID A IN HEALTHY AND OTOSTRONGYLUS-INFECTED JUVENILE NORTHERN ELEPHANT SEALS (*Mirounga angustirostris*)
Carolyn Cray,* Cole Kourgelis, Julie Sheldon, Cara Field, Shawn Johnson, and Nicole I. Stacy .................................................................................................................................... 198

EFFECTS OF INTRAMUSCULAR ALFAXALONE IN PERMIT (*Trachinotus falcatus*) AND SCHOOLMASTER SNAPPER (*Lutjanus apodus*)
Kathryn A. Tuxbury........................................................................................................................... 200

MAMMALS

CHOLECALCIFEROL SUPPLEMENTATION IN CAPTIVE ASIAN ELEPHANTS (*Elephas maximus*)
Sara E. Childs-Sanford, Rachel L. Hilliard,* Andrew J. Makowski, and Joseph J. Wakshlag ............................................................................................................................ 201

NEW WORLD SCREWWORM (*Cochliomyia hominivorax*) OUTBREAK AND TREATMENT IN ENDANGERED KEY DEER (*Odocoileus virginianus clavium*)
Lara Cusack,* Samantha Gibbs, Rebecca Shuman, and Mark Cunningham ........................................... 202

USE OF PALMAR DIGITAL NEURECTOMY TO MANAGE CHRONIC LAMENESS IN AN ADULT RETICULATED GIRAFFE (*Giraffa camelopardalis reticulata*)
Janis A. Raines,* Chris Bonar, Maren Connolly, and D. Reese Hand ..................................................... 204

IS IT TRUE THAT ELEPHANTS DON’T GET CANCER? LESSONS IN USING MORTALITY DATA
Carmel Witte, Allan Pessier, and Bruce Rideout* .................................................................................... 206

SERUM IMMUNOGLOBULIN E MEASUREMENTS AND CLINICAL RESPONSE TO ALLERGEN-SPECIFIC IMMUNOTHERAPY (ASIT) IN ASIAN ELEPHANTS (*Elephas maximus*)
Lydia Young,* Steven Scott, and Stephanie DeYoung .............................................................................. 207

SUCCESSFUL MANAGEMENT AND RESOLUTION OF A SEVERE NECROTIZING DERMATOPATHY IN A GOLDEN-HEADED LION TAMARIN (*Leontopithecus chrysomelas*)
Katharine Hope,* Tim Walsh, and Scott Norton ...................................................................................... 209
DISSEMINATED POLYSYSTEMIC LEIOMYOSARCOMA WITH INTRACRANIAL METASTASIS IN A MALE AFRICAN LION (Panthera leo)
Brian G. Stockinger,* Wm. Kirk Suedmeyer, Michael M. Garner, Matti Kiupel, and Brian Cellio ................................................................. 211

LUMBOSACRAL DISEASE IN A CAPYBARA (Hydrochoerus hydrochaeris)
Raphaëlle Boudreau,* Andreia F. Fernandes, Marion Desmarchelier, Andrea Matthews, Andrea Bourque, and Shannon T. Ferrell ......................................................... 212

OVIDUCT MORPHOLOGY AND PROLACTIN RECEPTOR EXPRESSION IN NORTH AMERICAN RIVER OTTERS (Lontra canadensis) AND ASIAN SMALL CLAWED OTTERS (Aonyx cinerea)
Rachel Melvin,* Anneke Moresco, Helen Bateman, and Dalen Agnew ......................................................... 213

BILATERAL ALVEOLAR OSTEITIS DUE TO TRAUMATIC AVULSION OF THE MAXILLARY CANINES IN A SUMATRAN ORANGUTAN (Pongo abelii)
Beth Westeren Romig* and Nikolay Kapustin ................................................................. 214
WHAT YOU SEE IS NOT WHAT YOU GET: ACCURACY OF NONINVASIVE ANESTHETIC MONITORING IN THE ANESTHETIZED GIRAFFE (Giraffa camelopardalis)

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Abstract

This study evaluated the accuracy of pulse oximetry, capnography, and oscillometric blood pressure during general anesthesia in giraffes (Giraffa camelopardalis). Thirty-two giraffes anesthetized for physiologic experiments were monitored using a pulse oximeter probe on the tongue and a capnograph sampling line at the distal end of the endotracheal tube. A blood pressure cuff was placed around the base of the tail, and an indwelling arterial catheter was placed in the auricular artery for blood gas analysis and invasive blood pressure measurements. Giraffes were intermittently ventilated using a Hudson demand valve throughout the procedures. Relationships between oxygen saturation as determined by pulse oximetry (SpO₂) and arterial oxygen saturation (SaO₂), between arterial carbon dioxide partial pressure (PaCO₂) and end tidal carbon dioxide (P(et)CO₂), and between oscillometric pressure and invasive arterial pressure were assessed, and the accuracy of pulse oximetry, capnography, and oscillometric blood pressure monitoring evaluated using Bland-Altman analysis. All three noninvasive methods provided relatively poor estimates of the reference values. As a novel approach, receiver operating characteristics (ROC) curve fitting was used to determine cut-off values for hypoxia, hypocapnia, hypercapnia, and hypotension for dichotomous decision-making. Applying these cut off values, provided reasonable sensitivity for detection of hypocapnia, hypercapnia, and hypotension, but not for hypoxemia. Noninvasive techniques currently available are not accurate in the anesthetized giraffe, and may produce “normal” readings in the face of serious abnormalities. As a consequence, noninvasive anesthetic monitoring results should be interpreted with great caution in giraffes and, ideally invasive monitoring should be employed.

Key words: Giraffa camelopardalis, giraffe, invasive monitoring, noninvasive monitoring
INTRAVENTOUS ANESTHESIA PROTOCOLS IN GREAT APES

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Abstract

Total or partial intravenous anesthesia (TIVA, PIVA) has been advocated in human and veterinary medicine as a potential means to improve hemodynamics and recovery times when compared to conventional inhalant anesthetic agents. In terms of great ape anesthesia, most veterinarians use a cyclohexanone/benzodiazepine or a cyclohexanone/α-2 agonist combination for induction followed by intubation and maintenance on either isoflurane or sevoflurane anesthesia. The use and benefits of TIVA and PIVA protocols in great apes has only been modestly described. Great ape anesthesia reports (n = 12) from two zoological institutions were evaluated for their TIVA/PIVA drug protocols and various simple indices. Species included gorilla (Gorilla gorilla, n = 8), bonobo (Pan paniscus, n = 1), and orangutan (Pongo pygmaeus, n = 1). Following induction with a cyclohexanone/benzodiazepine combination intramuscularly, patients were intubated and maintained on isoflurane (0.2-3%) and sevoflurane (0.5-2%) in 100% oxygen (n = 11 PIVA procedures), while one procedure was a complete TIVA procedure. Anesthesia was effectively and safely maintained using constant-rate infusions of a single agent or combinations of the following agents: fentanyl at 0.5-2.0 µg/kg/hr, remifentanil at 0.10-0.50 µg/kg/min, and propofol at 20-150 µg/kg/min. Heart rates were variable from 50-145 bpm. The longest procedure was a PIVA protocol that lasted 337 min with 135 min of intravenous infusion for anesthesia maintenance. Hypotension (systolic pressure less than 90 mm Hg) was the most common complication and was managed with increased fluid rates, the administration of glycopyrrolate, and vasopressor agents (dopamine or ephedrine) as needed. TIVA/PIVA provides an option for the maintenance of general anesthesia in great apes.

Key words: Anesthesia, CRI, great apes, hypotension, PIVA, TIVA
COMPARISON OF A GUAIFENESIN, KETAMINE, AND MEDETOMIDINE CONSTANT-RATE INFUSION WITH ISOFLURANE GAS FOR ANESTHESIA MAINTENANCE IN AMERICAN BLACK BEARS (Ursus americanus)

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Abstract

Seven American black bears (Ursus americanus) were anesthetized in a cross-over design with two different anesthetic maintenance protocols. The control protocol consisted of isoflurane gas started at 2%, and the experimental protocol consisted of a guaifenesin, medetomidine, ketamine (GMK) constant-rate infusion (CRI) started at a rate yielding 50 mg/kg/hr guaifenesin, 0.01 mg/kg/hr medetomidine, and 1 mg/kg/hr ketamine. A consistent induction protocol of 2 mg/kg ketamine and 0.04 mg/kg medetomidine was used in both protocols. Induction and recovery times including times of first effect, recumbency, and hands on; length of maintenance protocol; time from reversals administered to head up, to standing on all four feet, to no ataxia, and to fully recovered were recorded and compared between protocols. Heart rate, respiratory rate, rectal temperature, blood pressure, end tidal carbon dioxide, and hemoglobin oxygen saturation were recorded at 5-min intervals and compared between protocols. Venous blood gases taken at the beginning, end, and midway through the maintenance protocol were also collected and compared between protocols. All bears were mildly hypertensive with a mild respiratory acidosis. There were no statistically significant differences between the isoflurane and the GMK CRI maintenance protocols when comparing all other parameters measured with the exception of extubation times and endpoint pCO2 measurements. There were no adverse events recorded with either protocol, and adequate depth of anesthesia was maintained. A GMK CRI provides a safe and more portable alternative to inhalant anesthetics for maintenance anesthesia in bears in captivity or in the field.

Key words: American black bear, anesthesia, constant-rate infusion, guaifenesin, Ursus americanus

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MICROBIAL INTEGRITY STUDY OF PRESERVATIVE-FREE ALFAXALONE (ALFAXAN) IN A MULTI-USE SYSTEM WITH TWO STORAGE CONDITIONS AND THREE HANDLING TECHNIQUES

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Abstract

Alfaxalone (Alfaxan-CD, Jurox Pty Ltd.) is a neurosteroid that agonizes gamma-aminobutyric acid A (GABAA) receptors inducing anesthesia in numerous species when administered intravenously or intramuscularly.1 Alfaxalone has been reported to be a less favorable medium for bacterial growth when compared to propofol.2 The Australian Alfaxan-CD labeling permits broached vial storage at 4°C for up to 7 days, if contamination is avoided; however, the US Food and Drug Administration prohibits the use of Alfaxan beyond 6 hr because it is preservative-free. The objective was to evaluate the microbial integrity of preservative-free cyclodextrin-based alfaxalone (Alfaxan-CD) in a multi-use system, over 14 days, with two storage conditions (1. room temperature at 21°C and 2. refrigerated at 4°C) and three handling techniques (1. nonclosed dispensing port, 2. closed-system transfer device, and 3. vial stopper). Methodology comprised six treatment groups (n = 3 per group) and 0.5 ml per vial were withdrawn daily for 14 days. The samples were placed immediately in tryptic soy broth and sub-cultured onto sheep’s blood agar plates for objective evaluation of microbial growth. The results revealed no microbial growth in handling technique groups 1 and 2 at either storage condition, for up to 7 days. These findings support the Australian Alfaxan-CD labelled discard time of 7 days if stored at 4°C. Extending the discard time of Alfaxan minimizes drug waste and the subsequent environmental impact of unused drug, and offers a practical anesthetic alternative for use in veterinary practices across the United States.

Key words: Alfaxalone, Alfaxan, anesthesia, microbial integrity, preservative-free, storage

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LITERATURE CITED

PHARMACOKINETICS AND PHARMACODYNAMICS OF INTRAMUSCULAR ADMINISTRATION OF ALFAXALONE IN PEAFOWL (Pavo cristatus)

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Abstract

Alfaxalone, a neuroactive steroid with anesthetic properties, is considered safe used alone, or in combination with other drugs for anesthesia, and its use has been studied in numerous species. The objective of this study is to assess the pharmacokinetics and pharmacodynamics of intramuscular alfaxalone in Indian peafowl (Pavo cristatus). Eight female peafowl from the Cheyenne Mountain Zoo were used. A control blood sample was obtained prior to administration of either 10 mg/kg (n = 4) or 20 mg/kg (n = 4) alfaxalone, 10 mg/ml solution (Alfaxan®). Blood was collected at 5, 10, 15, 30 and 60 min post injection, with monitoring of sedation score, heart rate and respiratory rate at each time point. Peahens receiving a 10 mg/kg dose had smoother inductions and recoveries, though sedation level was generally scored as low, with no adverse reactions noted. All four birds in this group were considered fully recovered by the 60 min post-injection time point, although measurable alfaxalone plasma concentrations were present. All birds receiving 20 mg/kg experienced adverse effects including seizure-like episodes and hypersensitivity to stimuli throughout the study. This dosing group experienced prolonged recoveries consistent with high plasma concentrations (> 3000 ng/ml). Based on these findings, use of high doses of alfaxalone as the sole anesthetic agent is not recommended in this species; however, use in conjunction with other anesthetic/analgesic agents could be considered to allow for better sedation and smoother induction and recovery. Further research into combination inductions including alfaxalone and to assess pharmacokinetics and pharmacodynamics of alfaxalone in other avian species is needed.

Key words: Alfaxalone, Indian peafowl, Pavo cristatus, pharmacodynamic, pharmacokinetic

LITERATURE CITED


COMPARISON OF THE EFFICACY AND SAFETY OF MEDETOMIDINE-KETAMINE TO A NOVEL MEDETOMIDINE-AZAPERONE-ALFAXALONE COMBINATION IN WILD ROCKY MOUNTAIN BIGHORN SHEEP (Ovis canadensis)

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Abstract

Seventy-four wild, free-ranging Rocky Mountain bighorn sheep (Ovis canadensis) in Canada were darted intramuscularly (i.m.) with medetomidinea (0.15 ± 0.04 mg/kg) and ketamineb (4.0 ± 1.4 mg/kg) (MK, n = 37), or medetomidinea (0.14 ± 0.06 mg/kg), azaperonec (0.21 ± 0.11 mg/kg), and alfaxaloned (0.45 ± 0.21 mg/kg) (MAA, n = 37). Arterial blood samples were taken and analyzed immediately with a portable analyzer. Parameters evaluated were pH, PaCO2, PaO2, base excess, bicarbonate, oxygen saturation, and lactate. Rectal temperature, heart rate (HR), and respiratory rate were monitored upon recumbency and throughout anesthesia. Animals were reversed by i.m. injection of atipamezolee at five times the medetomidine dose. Induction times of animals darted once (8.7 ± 3.2 min, 7.3 ± 3.9 min) and recovery times of all animals (3.4 ± 1.5 min, 3.9 ± 1.6 min) were not significantly different between MK and MAA groups, respectively; however, MK animals had significantly longer downtimes (79.2 ± 10.5 min versus 52.2 ± 14.8 min, P < 0.0001). Both groups experienced severe hypoxemia (PaO2 41 ± 9 mm Hg, 40 ± 9 mm Hg). PaCO2 was significantly higher in the MK group than the MAA group (median 53 (range 52-56) mm Hg versus 48 (48-54) mm Hg, P = 0.0248), with a correspondingly lower pH (7.40 versus 7.42, P = 0.07). Initially, MK animals had significantly higher HR (49 versus 40 bpm, P = 0.0002), which decreased over time. In bighorn sheep, both MK and MAA produced reliable, reversible immobilization with rapid, smooth inductions and recoveries. MAA caused less respiratory depression than MK, and is a promising wildlife capture protocol.

aMedetomidine 30 mg/ml, Bow Valley Research Inc, Calgary, Canada
bVetalar® 100 mg/ml, Bioniche Animal Health Inc, Belleview, Canada
cStresnil® 40 mg/ml, Elanco, Division Eli Lilly Canada Inc, Guelph, Canada
dAlfaxan® 10 mg/ml, Jurox Pty Limited, Rutherford, Australia
eAtipamezole 10 mg/ml, Bow Valley Research Inc, Calgary, AB T2N 4G3, Canada

Key words: Alfaxalone, azaperone, bighorn sheep, ketamine, medetomidine, Ovis canadensis

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Animal Foundation First Award (Grant ID # D13ZO-317) to Åsa Fahlman, and a Natural Sciences and Engineering Research Council of Canada grant to Kathreen Ruckstuhl, University of Calgary. The authors also thank the University of Calgary R.B. Miller Field Station and volunteers assisting during field work.
EFFECTS OF COMBINING NITROPRUSSIDE WITH MEDETOMIDINE-AZAPERONE-ALFAXALONE IN CAPTIVE WHITE-TAILED DEER (*Odocoileus virginianus*)

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Abstract

Wildlife immobilization has experienced much refinement, but there is still room for improvement in induction time. Medetomidine causes profound vasoconstriction; this may limit its absorption locally and have an impact on induction time. This study aimed to determine if adding the vasodilator nitroprusside to medetomidine-azaperone-alfaxalone combination anesthesia resulted in a more rapid induction of anesthesia. In a crossover study, nine captive female white-tailed deer were hand-injected intramuscularly with either control (0.15 mg/kg medetomidine, 0.2 mg/kg azaperone, and 0.5 mg/kg alfaxalone) or treatment (0.07 mg/kg nitroprusside added) and released into an enclosure for observation; once recumbent, monitoring equipment was placed; arterial blood gas (ABG) was analyzed at 15 min post injection (PI); heart and respiratory rate, SpO₂, rectal temperature, and direct systolic, mean, and diastolic arterial blood pressures were recorded every 5 min; after 60 min PI, deer were reversed with intramuscular atipamezole (0.75 mg/kg). Statistical analysis was performed using ANOVA and descriptive statistics with a significance level of *P < 0.05*. Induction with nitroprusside (time to lateral recumbency 5.5 ± 3.0 sec) was not significantly different from control (7.1 ± 3.3 sec). Direct systolic, mean, and diastolic blood pressures were significantly lower with nitroprusside treatment. Interestingly, a significant improvement in oxygenation was observed with the nitroprusside treatment, as evidenced by an increased PaO₂ (52.8 ± 3.1 mm Hg for control; 61.4 ± 2.5 mm Hg for treatment) in ABG samples. Although nitroprusside induced no significant reduction in induction time, the significant increase in oxygenation is worthy of further investigation.

Key words: Anesthesia, hypoxemia, induction time, nitroprusside, *Odocoileus virginianus*, white-tailed deer
INVESTIGATIONS INTO THE USE OF PLETHYSMOGRAPHIC VARIABILITY INDEX (PVI) IN ANESTHETIZED CAPTIVE TIGERS (*Panthera tigris*)

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Abstract

Plethysmographic variability index (PVI) is an effective, noninvasive method to help evaluate central venous pressure and guide fluid therapy in humans and dogs.1,2 PVI is calculated via measuring dynamic changes in perfusion index during respiratory cycles, which mimics the amplitude of the pulse oximeter waveform. PVI changes also reflect vascular tone and circulating blood supply. The primary objective of this study was to investigate the applicability of PVI in monitoring anesthetized tigers (*Panthera tigris*). Eight adult, healthy tigers were anesthetized and mechanically ventilated. PVI, direct blood pressure (BP), capnography, and ECG were measured. Mean arterial pressure (MAP) was controlled and maintained at three different states (normotensive [MAP = 70 ± 5 mm Hg], hypotensive [MAP = 50 ± 5 mm Hg], and hypertensive [MAP = 90 ± 5 mm Hg]) for 20 min each, using changes in isoflurane concentration and administration of dobutamine. Arterial blood gas analysis was performed at the beginning of anesthesia and during each BP state. Mean PVI values were 8.44, 10.69 and 16.11 for hypertensive, normotensive, and hypotensive states respectively, and PVI in the hypotensive state was significantly different than PVI in normotensive and hypertensive states. PVI values were significantly correlated with MAP (r = 0.657, P < 0.0001), similar to the results of a hemorrhagic model in dogs.2 PaO2 values were greater (P < 0.05) in all BP states when compared to baseline and there were no differences in other blood gas variables amongst BP states. PVI is a highly portable, noninvasive method for predicting the BP status in tigers under general anesthesia.

Key words: Anesthesia, cardiovascular, *Panthera tigris*, plethysmographic variability index, tigers

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LITERATURE CITED


AVIAN INFECTIOUS DISEASES

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Abstract

Avian infectious diseases continue to be an important concern for human and avian health throughout the world. Many factors have and continue to make avian infectious disease a prevalent and ongoing concern including:

1. Birds harbor many pathogens which can affect not only humans but other mammals and multiple species of birds. These pathogens may not cause identifiable clinical signs.
2. Migration and flight facilitate infectious agent spread over large distances and from wildlife to captive populations.
3. Infectious diseases have caused extinction in avian species, especially island dwellers.
4. Habitat, ecosystem, and biodiversity degradation can increase human and likely other species’ exposure to infectious disease.
5. There is a lack of knowledge regarding prevalence and incidence of many avian diseases, and a lack of funding for continuing research of effective prevention strategies.
6. Avian management often is based on open systems.
7. Infectious disease testing is expensive, poorly comparable between labs, and may not be appropriate for use in a nontarget species.

Common infectious disease concerns among captive avian species, based on practical experience and from a consultation and management perspective, continue to be: chlamydiosis, avian influenza, West Nile virus (WNV), avian bornavirus, avian polyoma virus, and psittacine circovirus. This presentation will give a broad overview and then focus on diseases for which consults are commonly sought. Agents causing true zoonotic infections of birds and those proven to move from an infected bird to a human and cause disease, are few (Table 1). Generally they cause mild morbidity (not mortality) and are responsive to treatment. You should wear gloves, mask, and a lab coat and preferably work in a hooded space for all avian necropsies.

Potential zoonoses have been encountered in birds and occur in humans but the infectious routes have not been proven. Of particular importance are avian-associated diseases, as a source of confusion for many veterinarians and animal managers. Aspergillus spp. can cause acute fulminant disease in avian species but infected birds are not a source of disease for people. People are only subject to rare middle ear disease or bronchopulmonary allergy based on environmental exposure to this ubiquitous organism. Histoplasma spp. and Cryptococcus spp. grow in avian feces, not within the bird (one documented exception in a very sick, likely immunocompromised psittacine chick). Wear gloves and a mask and keep avian enclosures from becoming caked with feces and urates, but do not fear infection from bird contact based on this fungal contaminant. Secretion of
rabies virus has been documented to occur from a single great horned owl after experimental injection of rabies virus and steroids.

A number of encephalitis viruses that birds may carry usually require an insect vector (usually a mosquito) in order to be passed from the bird to a person. While WNV remains the hot topic, Eastern equine encephalitis (EEE), Western equine encephalitis (WEE), and Saint Louis encephalitis (STLE) viruses are all primarily carried by birds. The cycles for these viruses are between mosquitoes and passerine birds. Birds often have no or minimal clinical signs. Humans, horses, and small companion mammals are examples of dead-end hosts, which suffer disease and do not spread or amplify virus. Notable exceptions include alligators and crows and geese, which may spread WNV laterally without a vector. If sentinel for EEE or WEE was needed, an emu would be valuable as EEE and WEE may cause acute death, severe neurologic signs or hemorrhagic diathesis, but most people vaccinate emus for these diseases. These diseases are discoverable via antibody serology, PCR, or other viral assays; however, most diagnoses are determined postmortem. Raptors are generally recommended to be vaccinated and then have titers checked for WNV (National Veterinary Services Laboratory). Ratites are vaccinated for EEE and WEE.

While psittacosis remains the most commonly reported zoonotic disease of avian species, the authors receive few consultation calls regarding this bacterium. This is likely due to procedures that remain in place based on previous importation procedures for psittacines. Chlamydiosis is not only a disease of psittacine birds, but can affect all species and may have a prevalence approaching ~30% in free-living pigeons and doves. Recent public health reports from Sweden document increased cases of psittacosis based on bird feeder exposure of humans and free-living passerines. Chlamydiosis of raptor species is also now thought to be more common. Human-to-human transmission of psittacosis also is possible. A complete blood count serves as the initial screening test for psittacosis in the authors’ practice as infected birds often have leukocyte counts > 30,000 with a prominent lymphocytosis and or monocytosis (> 2000) in chronic stages of the disease. For case classification, further diagnosis and treatment, practitioners should be familiar with current recommendations from the compendium for chlamydiosis prevention and control.

Influenza (type A) remains a concern worldwide based on human health, mammal health and human food supplies (chickens, turkeys). As an RNA virus, avian influenza is one of the most rapidly evolving life forms on earth. Aquatic birds are the primary natural reservoir for most subtypes of influenza A viruses. Based on the vast silent reservoir in aquatic birds, influenza viruses cannot be eradicated. Most cause asymptomatic or mild infection in birds, where the range of symptoms depends on the viral properties. Viruses that cause severe disease in birds and result in high death rates (of chickens!) are called highly pathogenic avian influenza (HPAI). Recent examples include a Jan 15, 2016 HPAI H7N8 outbreak in commercial turkeys in Indiana and a March 5, 2017 HPAI H7N9 outbreak in commercial poultry in Tennessee, both of North American wild bird origin.

Viruses that cause outbreaks in poultry but are not generally associated with severe disease are called low pathogenic avian influenza (LPAI). No vaccine is currently approved for use in all avian species in the United States for influenza. Of the six licensed vaccines for poultry, label claims are limited to chickens and none provide adequate protection. The five steps for responding to an
outbreak continue to be: quarantine, eradicate, monitor region, disinfect, and test. As of 2016, based on requests for proposals to the private sector, multiple AI vaccines are either currently licensed or under development. For those under development, U.S. Department of Agriculture (USDA) is working closely with the manufacturers to expedite the review and approval of products to ensure quick availability. The USDA then intends to stockpile vaccine as protection during an outbreak. The vaccination would be a suppressive emergency approach, where commercial poultry in a defined geographic area with rapidly spreading disease would be vaccinated.

Influenza viruses that humans can contract include avian influenza virus subtypes A (H5N1), A (H7N9), and A (H9N2) and swine influenza virus subtypes A (H1N1) and (H3N2). Most human cases of A (H5N1) and A (H7N9) infection have been associated with direct or indirect contact with infected live or dead poultry. For swine influenza, close proximity to infected pigs or visiting locations where pigs are exhibited has been reported for most human cases; some limited human-to-human transmission has occurred. Most human influenza infections are from direct contact or contaminated environments. Subsequent transmission to other people is inefficient. To minimize public health risk, surveillance of animal and human populations is essential.

Avian bornaviruses are widespread and may affect multiple species (Table 2). In many cases, infection may have minimal clinical effects. Much basic information regarding pathogenesis, infective route, disinfection and treatment remain to be elucidated. No bird should be euthanized based on a single positive PCR test for avian bornavirus. No avian bornavirus has caused documented infection or disease in humans; however, recent bornaviral infection in tree squirrels have caused encephalitis and death in humans.

**Key words:** Biodiversity, bornavirus, chlamydiosis, influenza, management, parrot, passerine, waterfowl, zoonoses

**LITERATURE CITED**


Table 1. Bacterial, fungal, viral, and parasitic etiologies of zoonotic concern of birds.

<table>
<thead>
<tr>
<th>Zoonoses</th>
<th>Potential zoonoses</th>
<th>Avian-associated disease</th>
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<tr>
<td>Psittacosis&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Mycobacteriosis&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Aspergillus&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Pseudotuberculosis&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Other bacteria&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Newcastle’s disease&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Avian influenza&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup>Bacterial  
<sup>b</sup>Fungal  
<sup>c</sup>Viral  
<sup>d</sup>Parasitic

Table 2. Recent bornaviruses reclassification.

<table>
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<th>Family</th>
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<td>Mammalian 1 bornavirus</td>
<td>Borna disease virus 1 (BoDV-1)</td>
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<td>Borna disease virus 2 (BoDV-2)</td>
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APPENDICULAR FRACTURES IN BIRDS OF PREY: A RETROSPECTIVE STUDY, 2000-2015

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Abstract

Trauma is the most frequent cause of admission of free-living birds of prey to rehabilitation centers; however, few studies have described the prevalence and prognosis factors of appendicular fractures in birds of prey. This retrospective study evaluated potential risk factors of open appendicular fractures in birds of prey and investigated prognostic factors for appendicular fractures such as species and fracture characteristics. Outcomes are described based on comparison of surgical versus conservative approaches. Birds were recruited after initial triage. Logistic regression models were used. From 2000 to 2015, 534 birds of prey presented at the Clinique des Oiseaux de Proie (COP) fulfilled inclusion criteria to evaluate potential risk factors of open appendicular fractures (first objective) and 121 birds fulfilled inclusion criteria to investigate appendicular fractures prognosis factors (second objective). Risk factors identified for open fractures in raptors included larger birds and bones with less soft tissue coverage, which appeared more prone to open fractures. Birds treated for open fractures had a release rate (n = 24/52, 46%) similar to birds presented with closed fractures (n = 32/69, 46%) based on triage criteria (euthanasia of birds presenting extensive devitalized exposed bone, lack of pain perception associated with nerve damage, myasis association with extensive infection, complete tendinous rupture, complex articular fractures and exposed joints) described in the present study. This may result from efficient triage criteria for open fractures that are enforced at the COP. Birds presented with open appendicular fractures should not be systematically euthanized as is sometimes advocated.

Key words: Open fracture, prognosis, raptor, release rate, risk factor, surgery

ACKNOWLEDGMENTS

The authors would like to acknowledge the interns and residents of the Service de médecine zoologique, and the Union Québécoise de Réhabilitation des Oiseaux de Proie (UQROP) for their implication in this study and the countless volunteers and students of the Université de Montréal helping in the Raptor rehabilitation program.

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Abstract

Although liver disease is common among companion psittacine birds, the diagnosis of hepatic disease is challenging. Clinical signs are often nonspecific, hepatocellular leakage enzymes are not exclusive to hepatocytes, and up to 80% of hepatic tissue must be dysfunctional before clinical signs of hepatic failure become apparent. The correlation of plasma biochemistry, hematologic, radiographic, and endoscopically observed gross abnormalities with histologically confirmed hepatic pathology have not been extensively studied. A retrospective study was conducted from medical records of psittaciformes at a veterinary teaching hospital from 2007 through 2016. Plasma biochemical, radiographic, and endoscopic visualization findings were classified as abnormal or normal. Each of these findings were compared to hepatic histopathology for agreement or disagreement. Statistical significance was based on the Kappa test for agreement and McNemar’s test for disagreement. On histopathology, 25 of 28 psittacine birds had liver lesions. Only 11 of 28 psittacine birds (39.3%) had clinical signs considered specific for hepatic disease. Gross changes on radiography or endoscopy agreed with histopathology in 57.1% and 53.6% of cases, respectively. Aspartate aminotransferase (AST) (48.1%), creatine phosphokinase (CPK) (73.1%), and albumin by protein electrophoresis (50%) showed highest agreement. No parameter had significant agreement while disagreement reached significance for radiography, endoscopy, AST, lactate dehydrogenase (LDH), bile acids, total protein, cholesterol, triglycerides, uric acid, and glucose. Abnormalities of plasma biochemistry, hematology, radiography, and endoscopy are nonspecific and do not consistently agree with histopathology results. Reliable predictive value is lacking. Endoscopic liver biopsy is recommended to confirm clinical suspicion of liver disease.

Key words: Avian, diagnosis, hepatic disease, liver disease, parrot, psittaciforme

Acknowledgments

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HEMOPARASITE INFECTIONS IN SNOWY OWLS (Bubo scandiacus)

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Abstract

Hemoparasites, including Plasmodium, Leukocytozoon and Haemoproteus spp., are a common finding on blood smears of various avian species. Wild passerine birds often serve as reservoirs for these vector-borne hemoparasites, and most hemoparasites found in raptor species are associated with subclinical infection rather than overt disease.²,⁴-⁵ Very few documented reports of hemoparasite associated disease in raptors exist.¹,³,⁶ This case series describes five cases of hemoparasite infection in snowy owls (Bubo scandiacus). Of these cases, one mortality was attributed to hemoparasite infection, and Plasmodium spp. infection contributed to the death of a snowy owl in another case. The remaining three infected snowy owls were treated with primaquine⁴ (1 mg/kg every 7 days for five doses, then s.i.d. for 10-15 doses p.o.) and chloroquine⁵ (10 mg/kg loading dose, then 5 mg/kg s.i.d. for three doses, then every 7 days for four doses, then s.i.d. for 10-15 doses p.o.) and survived the infection. Based on serial blood smear monitoring, hemoparasite burdens decreased with treatment. Hemoparasitic infection does not appear to be benign in snowy owls as it is considered to be in other raptor species. Increased environmental temperatures, stress, novel pathogen exposure, and concurrent disease processes may contribute to this morbidity and mortality in this species.

Environmental changes including the addition of fans to cool the exhibit and improved vector control have been implemented. Over the subsequent 2.5 yr, lower parasite burdens have occurred on blood smears and owls have remained asymptomatic without administration of anti-malarial medications.

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Key words: Chloroquine, Haemoproteus, Leukocytozoon, mortality, Plasmodium, primaquine

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PATHOGEN PREVALENCE IN THE YELLOW-EYED PENGUIN (*Megadyptes antipodes*)

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Abstract

The yellow-eyed penguin (*Megadyptes antipodes*) is an endangered species endemic to New Zealand that has faced a number of significant mortality events in recent years, and is composed of two distinct and isolated population centers. Previous research has suggested that the differing ecosystems of the distinct population centers may influence the incidence of pathogens. The research aims to develop insights into host-pathogen relationships of wildlife disease in this endangered species, and examine the risk of novel pathogens being introduced into the fragile populations of this endangered species. Thus the prevalence of known pathogens including *Plasmodium* spp. and *Eimeria* spp. within these meta-populations was studied. Surveys of the wild population centres and birds held in rehabilitation facilities revealed significant differences between sample groups. Blood samples collected in 2014 from penguins in rehabilitation were 66% (*n* = 45) positive for *Plasmodium* spp. on PCR; however, only one of the 18 wild birds sampled was positive for *Plasmodium* spp. Of the wild penguins sampled from the subantarctic in 2016, all birds were negative for *Plasmodium* spp. (*n* = 65) but showed a high prevalence (76.6%) of coccidial (*Eimeria* spp.) oocysts (*n* = 47).

Key words: Avian malaria, coccidian, disease, *Eimeria, Plasmodium*, Sphenisciformes, Subantarctic

ACKNOWLEDGMENTS

The authors would like to thank the Department of Conservation, Yellow-Eyed Penguin Trust and Massey University for their assistance with this research.

LITERATURE CITED


REVIEW OF MORTALITY AND EFFECTIVENESS OF NEONATAL TREATMENT IN CAPTIVE ATTWATER’S PRAIRIE CHICKENS (Tymanuchus cupido attwateri)

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Abstract

A retrospective study of mortality and neonatal treatment of 975 captive Attwater’s prairie chickens less than 8 wk old at the Houston Zoo was conducted from 2009-2015. Birds were reared in communal groups and moved sequentially from brooders to juvenile cages (both with reusable substrate matting) to outdoor pens with nonorganic substrate (gravel) prior to transport to pre-release sites.¹ Diet included a custom formulated dry meal,a greens, and small insects.⁵ Chick mortality rate was 36% (n = 352) with over 75% of all deaths occurring before 10 days of age. Severe weight loss that required treatment was associated with a significantly reduced chance of survival (P < 0.0001); however, treatment with gavage feeding of a highly digestible formula (15 ml/kg p.o. b.i.d. into the crop)b-d had no effect on mortality rates (47%; n = 154) (P = 0.4005).⁶ The most common cause of death was bacterial gastrointestinal disease (44%; n = 156) including yolk sac infection, necrotizing enteritis, and mucoid enteritis. Gavage feeding correlated with a significantly higher proportion of necrotizing enteritis (n = 32) (P = 0.0003). Necrotizing enteritis has been associated with Clostridium perfringens type A infection and is transmitted via the fecal-oral route. Limiting fecal contamination is imperative to reducing transmission of clostridial spores.³⁴⁷ Treatment with meloxicam (0.2 mg/kg s.i.d.-1 mg/kg s.i.d.)e was associated with a significantly reduced proportion of mucoid enteritis (P = 0.0483). Mortality due to avian pathogenic Escherichia coli yolk sacculitis is a point of future investigation for this species.²

Key words: Attwater’s prairie chicken, chick mortality, enteritis, neonatal treatment, Tymanuchus cupido attwateri, yolk sac infection
ACKNOWLEDGMENTS

The authors would like to thank the dedicated staff of the bird department of the Houston Zoo, the pathology department of Texas A&M Veterinary Medical Diagnostic Laboratory, and the U.S. Fish and Wildlife Service staff at the Attwater’s Prairie Chicken National Wildlife Refuge for their years of service to the APC captive breeding program.

LITERATURE CITED


PHARMACOKINETICS OF A CONCENTRATED BUPRENORPHINE FORMULATION IN RED-TAILED HAWKS (Buteo jamaicensis)

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Abstract

Opioid drugs are the cornerstone therapy for moderate to severe pain management, and recent studies have shown mu opioid agonists to be effective in some raptor species.1-3 The pharmacokinetics of two doses of a concentrated formulation of buprenorphine (Simbadol™, 0.3 mg/kg s.c.)a were evaluated in red-tailed hawks (Buteo jamaicensis, n = 6). Blood samples were collected at 10 time points over a 24-hr period after drug administration to determine buprenorphine plasma concentrations using liquid chromatography-tandem mass spectrometry. After a 4-wk washout period, the same birds were administered 1.8 mg/kg s.c. of the same formulation. Blood samples were collected at 13 time points over a 96-hr period after drug administration. The hawks were monitored for adverse effects and assigned a sedation/agitation score at each time point. Maximum buprenorphine concentration was achieved at 5 and 15 min for the 0.3 mg/kg and 1.8 mg/kg doses, and plasma concentrations were maintained above 1 ng/ml for at least 24 and 48 hr, respectively. The elimination half-life was 7.12 and 8.74 hr for the low and high doses. There were significantly higher sedation scores for both doses (P < 0.001) at the time points evaluated between 15 min and 48 hr. No adverse effects were noted in any birds. In summary, the formulation evaluated results in rapid absorption of buprenorphine and plasma concentrations above 1 ng/ml for 24 and 48 hr following administration of 0.3 and 1.8 mg/kg s.c. respectively, causing mild to moderate sedation in red-tailed hawks.

aSimbadol™ 1.8 mg/ml buprenorphine, Zoetis, Parsippany, New Jersey 07054 USA

Key words: Analgesia, buprenorphine, Buteo jamaicensis, pharmacokinetics, red-tailed hawk, Simbadol

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MINIMIZING RISK BY MAXIMIZING INTERVENTION TO PREVENT SALMONELLA IN AVIARY RAINBOW LORIKEETS (Trichoglossus haematodus)

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Abstract

The San Diego Zoo Safari Park walk-through aviary of rainbow lorikeets (Trichoglossus haematodus) has a history of morbidity and mortality caused by Salmonella enterica ser. Typhimurium. A 3-mo long epornitic in 2014 affected 15 birds (21%) with high case mortality (53%) and sporadic cases over the next year. All 57 birds were evaluated and those with clinical signs or laboratory evidence of disease were individually treated. Monocyte count, fecal PCR, and Salmonella titers were most effective at diagnosing subclinical disease and monitoring response to treatment (enrofloxacin 20 mg/kg p.o., s.i.d. for 21 days). Serology was also used to monitor response to vaccination with an inactivated Salmonella spp. bacterin.1 In 2017, all aviary birds (n = 51) received the first dose subcutaneously and a second dose orally after 4 wk. Serology prior to vaccination showed resolution of previously high titers from infection in most birds. Post-vaccine, opportunistic titers were measured to evaluate response to vaccination and to develop an effective vaccine protocol. While humoral immune response post vaccination has been inconsistent, no adverse reactions were noted. Early intervention and stream-lined intervention protocols have reduced death due to salmonellosis, which has been the cause of death in only one individual (4%) since 2015. Husbandry staff also changed feeder design and heightened vigilance to hygiene and bird behavior. Since implementation of targeted flock surveillance and husbandry modifications to minimize disease risk and maximize early intervention, salmonellosis morbidity and mortality have been drastically reduced.

Key words: Avian, Salmonella, serology, titer, vaccine, zoonosis

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Special thanks to Dr. Spencer Kehoe for his early work on this outbreak and its followup, and to Dr. Bran Ritchie for his invaluable work to develop the vaccine and consult on multiple aspects of this management. The staff at the Wildlife Disease Laboratory and the San Diego Zoo Safari Park Veterinary Services and Bird Department (veterinarians registered veterinary technicians, hospital keepers, and bird keepers) deserve significant recognition for their dogged pursuit of answers and dedicated treatment and work on these cases.
STANDING COMPUTED TOMOGRAPHY IN NONANESTHETIZED LITTLE PENGUINS (Eudyptula minor) TO ASSESS NORMAL ANATOMY AND MONITOR DISEASE

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Abstract

Computed tomography (CT) is increasingly available for zoo species to aid in health assessment. Best CT interpretation is facilitated by establishment of species-specific CT baselines in health. Multi-detector CT (MDCT) provides high quality images in a short time, eliminating the need for anesthesia in some patients.² MDCT scans were performed in clinically healthy, nonanesthetized, standing little blue penguins (Eudyptula minor) to establish normal reference ranges for air sac and lung volumes, and lung density. Unexpectedly, five out of 15 clinically normal penguins were diagnosed with pulmonary granulomas on initial MDCT scans. Granulomas were not visible on radiographs, even in cases where the entire normal pulmonary parenchymal architecture was effaced on the MDCT scan. In one penguin with suspected aspergillosis, granuloma regression was documented on serial MDCT scans after treatment with antifungal medications; however, follow-up MDCT scans in the remaining four penguins with granulomas revealed progression in granuloma size and numbers. Additional testing led to the diagnosis of mycobacteriosis in multiple birds. Serial MDCT scans in this cohort enabled detection of lesions prior to development of clinical disease and facilitated adequate localization for fine needle aspiration of granulomas. Mycobacteriosis treatment was initiated early, and response to therapy was monitored by serial MDCT scans with minimal patient risk. Based on experience, nonanesthetized MDCT scans are a well-tolerated, noninvasive test modality that provides real-time results for otherwise difficult to diagnose diseases such as aspergillosis and mycobacteriosis in some penguins.¹³

Key words: Aspergillosis, imaging, multi-detector computed tomography, mycobacteriosis, pulmonary granuloma, Sphenisciformes

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The authors thank Raphael Costa, LVT for his expertise in operating the CT scanner and essential assistance in obtaining CT scans of the birds. Additionally, authors are grateful to the Radiation Oncology Department at the Animal Medical Center for generously allowing use of their radiation planning software for volumetric and density measurements. Finally, authors thank the Ornithology Department at the Bronx Zoo, particularly supervisor Susan Schmidt, for their care of the birds and for their assistance in completion of this project.

LITERATURE CITED


EFFECTS OF MIDAZOLAM AND MIDAZOLAM-BUTORPHANOL SEDATION ON GASTROINTESTINAL TRANSIT TIMES IN COCKATIELS (*Nymphicus hollandicus*).

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Abstract

Gastrointestinal (GI) contrast radiography studies are frequently performed in birds and provide valuable diagnostic information. Sedation in birds is commonly performed in order to facilitate a variety of diagnostic and therapeutic procedures and therefore may facilitate repeated radiographic positioning for GI contrast studies; however, the effects of sedative drugs on GI motility and transit times have not been evaluated in birds to date. A controlled, randomized, blinded, complete cross-over study of 12 healthy adult cockatiels (six male, six female) (*Nymphicus hollandicus*) was used to evaluate the effects of midazolam (6 mg/kg i.m.), and midazolam (3 mg/kg i.m.) combined with butorphanol (3 mg/kg i.m.) on GI transit times and motility. Iohexol (20 ml/kg) was administered by crop gavage 15 min after administration of the sedative drugs and fluoroscopy was used to evaluate gastrointestinal transit times and motility at several times points. Both sedation protocols significantly affected GI transit times and motility (*P* < 0.05) and the MB protocol had more pronounced effects. Overall median (range) GI transit times were 60 (30-120), 90 (30-120) and 120 (120-180) min for the control, M, and MB groups, respectively. Ventricular contractions were markedly reduced with both sedation protocols, while esophageal boluses were only reduced in the MB group. The results of this study show that commonly used sedative drugs have a significant effect on GI transit time and motility in birds. This effect should be considered when planning and interpreting GI contrast studies in avian patients.

**Key words:** Benzodiazepine, chemical restraint, contrast study, motility, opioid, radiography

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INVERTEBRATE PHARMACOLOGY

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Abstract

Invertebrate pharmacology with regard to veterinary medicine and health management is still in its early stages. Much of how veterinarians practice in this area is based on experience, anecdotal information, and clinical trials. Most of the pharmacokinetic data available pertains to just a few taxa (honey bees, horseshoe crabs, decapod crustaceans, and certain mollusks). These notes contain doses and references for this latter group and some other information based on information in the peer-reviewed literature, and in some cases, review articles and textbooks.

Key words: Anesthetics, antimicrobials, invertebrates, parasiticides, pharmacology

INTRODUCTION

Invertebrate animals comprise over 95% of the animal kingdom’s species, yet nonparasitic invertebrates are vastly underrepresented in the typical veterinary school curriculum, and relatively little is known about therapeutic pharmacology for this immense number of species, who all have just one thing in common: they lack a vertebral column. In fact, many invertebrates are more closely related to some vertebrates than each other.

These notes and the accompanying lecture provide a brief introduction to some of the more prominent invertebrate groups (coelenterates, mollusks, crustaceans, spiders, horseshoe crabs, insects, and echinoderms) and review the state of the science with regards to chemotherapeutics and pharmacokinetics. This manuscript is by no means comprehensive, and is primarily meant to inform the interested clinician on the possibilities related to working with drugs and invertebrates, and the most current publications in this area.

COELENTERATES

This large phylum includes the comb jellies (ctenophores), Hydrozoans (hydoras, fire coral, Portuguese Man-O-War), Scyphozoans (jellyfishes), and Anthozoans (stony corals, soft corals, sea anemones). This is an economically important group for research, environmental monitoring, public and private display, and tourism. Coral reefs collectively are one of the most beautiful, diverse, and fragile ecosystems on the planet. Jellyfish exhibits are now some of the most popular displays in public aquariums and upscale restaurants throughout the world.

Antimicrobials

Ampicillin. Used to control white band disease in Acropora at a dose of 100 mg/L every 12 hr for 7 days.40
Chloramphenicol. Used as an immersion at 10-50 mg/L for several days with a 100% water change every 24 hr. This regimen is not based on a pharmacokinetics study; the values are taken from review articles and textbooks. 10,38,39

Lugol’s iodine (5%). Used as an immersion for corals and anemones. It is an antiseptic that will cauterize wounds. It is a strong oxidizing agent and some corals are sensitive, including Xenia sp., Anthelia spp., and Pachyclavularia spp. If corals appear stressed then remove immediately. 39

Paromomycin. Used to control white band disease in Acropora at a dose of 100 mg/L every 12 hr for 6 days. 40

Tetracycline. Used for bacterial infections as a 10 mg/L immersion. No pharmacokinetics data and positive results are anecdotal. 30,39

**Parasiticides**

Diflubenzuron. To control parasitic amphipods of the jellyfish, Chrysaora sp. The dose is 0.03 mg/L for 7 days. 17

Freshwater. A 1-3 min dip is commonly used to treat for ectoparasites such as turbellarians (flatworms). Caution must be taken to make sure the freshwater is pH matched to the seawater. Corals (hard and soft) should be monitored closely, and when possible, biotest with one small or less valuable specimen first. 39

Levamisole. Used as an immersion for helminths at 8 mg/L for 24 hr. This regimen is well tolerated by many species of stony corals. 39

Milbemycin oxime. Used as a treatment for “red bug” on Acropora corals. Red bugs are actually the copepod Tegastes acroporanus. The dose is 0.167 µg/L of the otic solution (MilbeMite®) for 6 hr. 15

**GASTROPOD MOLLUSKS**

The gastropods belong in the phylum Mollusca and include over 80,000 marine, fresh water and terrestrial species. All gastropods have a ventrally flattened foot that provides locomotion along the various surfaces of their habitats. The group includes snails, slugs, sea hares, nudibranchs, slipper shells, conchs, whelks, and abalone, among many others. The use of gastropods as laboratory animals and in aquaculture is limited but does occur. They are important display and food animals. Investigators working on the sea hare, Aplysia, were awarded a Nobel Prize for medicine or physiology in 2000.

**Anesthetics**

Clove oil (Eugenol). Used for apple snails at 0.35 g/L as an immersion. 20
Ethanol (5%). Has been used as an immersion anesthetic for aquatic gastropods including abalone.\textsuperscript{26}

Ethanol/methanol (10%). The trade name for this compound is Listerine\textsuperscript{®} and it has been used in cultured \textit{Lymnaea} to effect.\textsuperscript{47}

Magnesium chloride. Can be used as an immersion (normally 7.5%) in many aquatic gastropods. Queen conchs have been anesthetized with 30 g/L.\textsuperscript{1}

Magnesium sulfate. Used in abalone at between 4 and 22 mg/L as an immersion.\textsuperscript{46}

Sodium pentobarbital. Can be used at 1 ml/L for abalone to effect or 400 mg/L to effect. Note this is a controlled drug.\textsuperscript{5}

**CEPHALOPOD MOLLUSKS**

There are about 650 species of cephalopods, a taxon that includes the octopuses, squids, cuttlefish, and the chambered nautilus. This is an important economic group in that they serve as a food source for humans and other animals, are popular display animals, and have been frequently employed in a variety of research projects. Their acute vision, manual dexterity, and intelligence make them fascinating animals to observe and study. Unfortunately, most species are short-lived in the wild and captivity.

**Antimicrobials**

Enrofloxacin. The dose is 5 mg/kg IV, 10 mg/kg PO, and 2.5 mg/L as a 5-hr immersion. Dosing interval has not been worked out and these values are from the European squid.\textsuperscript{25}

**Anesthetics**

Ethanol. Has been used as an immersion anesthetic in many cephalopods at a variety of concentrations from 1.5-10\%.\textsuperscript{24,27}

Magnesium chloride. Used in European cuttlefish at 6.8 g/L\textsuperscript{25} and 32.5 g/L in octopus.\textsuperscript{24}

**BIVALVE MOLLUSKS**

This class of mollusks contains many common animals including the clams, mussels, oysters, and scallops. This is an extremely economically important group, especially as a food source for humans. Many species are both captured and cultured for food worldwide. There are more than 10,000 recognized species, found in freshwater, estuarine and marine surface waters. Bivalves fill a critical niche within aquatic ecosystems, the majority functioning as living filters. They comprise a large portion of the shell fauna collected by amateur or professional conchologists on beaches and freshwater stream banks, and historically have played a significant role in the apparel industry as a source of buttons, or pearls, and as a frequent item on the shelves of novelty shops. Bivalves are popular in display aquariums (private and public aquaria) and as research animals.
**Antimicrobials**

Enrofloxacin. Used as a 5 mg/L immersion in Manila clams.\(^{14}\)

**Anesthetics**

Propylene phenoxytel. Use 1-3 ml/L of a 1% solution for oysters.\(^{32}\)

**CRUSTACEANS**

The crustaceans are a highly successful class of the Phylum Arthropoda. This group includes the well-known lobsters, crabs, crayfish, shrimp, barnacles, and hermit crabs. Numerous other taxa belong to this class including isopods, amphipods, and brine shrimp. Economically, this is one of the most important groups of invertebrates. Its members are important for food, research, and as display animals.

**Antimicrobials**

Enrofloxacin. The dose is 10-20 mg/kg i.m. for Chinese mitten crabs.\(^{41}\)

Oxolinic acid. Given intrasinus at a dose of 10 mg/kg for Kuruma shrimp.\(^ {42}\)

Oxytetracycline. Given intrasinus at 25 mg/kg in Kuruma shrimp,\(^ {42}\) 10 mg/kg intrasinus in tiger shrimp,\(^ {43}\) and 50 mg/kg p.o. in both species.\(^ {43}\) For Gaffkemia (*Aerococcus viridans*) in American lobsters use 2.2 mg/g of food.\(^ {9}\)

Sulfadimethoxine. For American lobsters the dose is 42 mg/kg intrapericardial.\(^ {8}\)

Sulfamethoxazole trimethoprim. For bioencapsulation in brine shrimp. Use toe tread white shrimp by combining 20-40% trimethoprim sulfamethoxazole with a lipid emulsion (Selco, INVE Aquaculture) at a concentration of 1:5.\(^ {13}\)

**Anesthetics**

Clove oil (Eugenol). Approximately 125 mg/L as an immersion.\(^ {19}\)

Ketamine. Can be used at 1 mg/kg i.m. for Australian giant crabs.\(^ {19}\)

Lidocaine. Given to shrimp i.m. at 0.4-1 mg/kg.\(^ {11}\)

Xylazine. For giant Australian crabs at a dose of 16-22 mg/kg i.v.\(^ {19}\)

**SPIDERS**

This is a huge group of animals (over 30,000 species) that belong to the class Arachnida. Less conspicuous arachnids include the mites, ticks, and scorpions. Numerous texts describe the
biology, natural history, and husbandry of these fascinating creatures. Tarantulas (not true spiders) represent an important group of commonly kept arachnids that occasionally require medical care.

**Anesthetics**

Alfaxalone. Given to tarantulas at a dose of 200 µg/kg intracardiac.\(^{23}\)

Carbon dioxide. Normally used in an anesthetic chamber at 3-5%.\(^{26}\)

Isoflurane. Normally used in an anesthetic chamber at 5% with 1 L/min oxygen flow\(^{18,49}\) but can be used with a cotton ball with adequate protection/separation for the spider.\(^{6}\)

Ketamine. Given intracardiac at 20 mg/kg along with 200 µg/kg alfaxalone.\(^{23}\)

Morphine. Given intracardiac at 5 mg/kg along with 200 µg/kg alfaxalone.\(^{23}\)

Sevoflurane. Normally used in an anesthetic chamber at 5% with 1 L/min oxygen flow.\(^{50}\)

Xylazine. Given intracardiac at 20 mg/kg along with 200 µg/kg alfaxalone.\(^{23}\)

**HORSESHOE CRABS**

*Limulus polyphemus*, the American horseshoe crab, is actually not a crab at all but a member of the Class Merostomata in the Phylum Chelicerata. Horseshoe crabs are more closely related to arachnids than crustaceans. This is the only species that lives on the Western Atlantic coast, but there are other species of horseshoe crabs that occur in Asia. *Limulus* is a very important animal for biomedical research and is used as bait and fertilizer (controversial) as well as being an important display and touch tank animal in public aquaria. Investigators examining vision and the *Limulus* lateral eye were awarded the Nobel Prize for medicine or physiology in 1967.

**Antimicrobials**

Itraconazole. Given i.v. at a dose of 10 mg/kg.\(^{3}\)

Oxytetracycline. The dose is 25-50 mg/kg i.v.\(^{33}\)

**INSECTS**

This is by far the largest group of invertebrates and possibly the most economically important. Insects are loved and despised worldwide and occupy nearly all niches except the marine environment. They are important as a human food source in parts of the world and both sustain and destroy agricultural crops, depending on the species of insect and plant. Much of the veterinary-pertinent literature focuses on the honeybee (*Apis mellifera*).
Antimicrobials

Oxytetracycline. For treatment of American and European foulbrood in honeybees at a dose of 200 mg/colony every 4-5 days for three treatments. Note, there is a lot of resistance now. Those treating honeybees need to recognize they are considered food animals in the United States and proper Veterinary Feed Directive measures need to be followed.

Tylosin. For treatment of American foulbrood in honeybees at a dose of 200 mg/colony every 4-5 days for three treatments. Those treating honeybees need to recognize they are considered food animals in the United States and proper Veterinary Feed Directive measures need to be followed.

Parasiticides

Amitraz. For control of mites (acarasis) in honeybees. Use as directed but realize many factors are involved with a hive mite infestation.

Formic acid. For control of mites (acarasis) in honeybees. Commercial packaging should be consulted prior to use.

Fumagillin. For control of nosemosis (a microsporidian parasite) in honeybees. Use as directed and consult packaging.

Menthol. For control of mites (acarasis) in honeybees. Commercial packaging should be consulted prior to use.

Thymol. For control of mites (acarasis) in honeybees. Commercial packaging should be consulted prior to use.

ECHINODERMS

This interesting and diverse group of animals includes the sea stars, brittle stars, sea cucumbers, sea urchins, sea biscuits, and crinoids (feather stars). Many are commonly displayed in aquaria and used in research. Humans do not consume most species but the gonads of sea urchins and the tunic of sea cucumbers are popular fare in some countries and restaurants in the United States that serve sushi.

Antimicrobials

Enrofloxacin. For green sea urchins, 10 mg/kg ICe or 10 mg/L as a 6-hr immersion. For purple sea stars, 5 mg/kg ICe or 5 mg/L as a 6-hr immersion.

Oxytetracycline. For chocolate chip sea stars, 10-15 mg/L immersion every 48-72 hr for three to five treatments.
Tris EDTA and neomycin (Tricide-Neo®). For cushion sea stars with cutaneous ulcers, use 100 ml/L for 45 min every 24 hr for 7 days.  

**Anesthetics**

Magnesium chloride. Use a 1:1 mixture of seawater and 7.5% MgCl₂.  

Propylene phenoxetol. For sea cucumbers use 2 ml/L to effect.  

Tricaine methanesulfonate (MS-222). For purple sea urchins, 0.4-0.8 g/L immersion to effect.  

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HEMOLYMPH CYTOLOGY, CELL COUNT, AND ELECTROLYTE REFERENCE VALUES IN CAMEROON RED TARANTULA (Hysterocrates gigas)

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Abstract

Theraphosidae is a large family of tarantula spiders commonly kept as pets or display animals in zoological institutions. A variety of diseases and diagnoses have been reported in captive tarantulas. There has been a growing interest and demand over the last decade for arachnid medicine, but the range of currently available diagnostic tests is limited. Hemolymph analysis could be a fundamental tool for arachnid health assessment. The objective of this prospective study was to establish reference intervals for hemocyte counts, and acid-base and electrolyte parameters in Cameroon red tarantulas (Hysterocrates gigas). Ninety-nine mature Cameroon red tarantulas from the Toronto Zoo collection were anesthetized using isoflurane in an induction chamber for the collection of 0.35 ml of hemolymph from the dorsal opisthosoma. Electrolytes (sodium, chloride, potassium, ionized calcium, and bicarbonate) were measured using a radiometer (ABL800 FLEX, Radiometer America, Brea, California 92821 USA), and hemocyte counts were obtained using the Avian Leukopet™ system (Vetlab Supply, Palmetto Bay, Florida 22157 USA) and hemocytometer. Cytocentrifuge preparations of hemolymph were Wright’s stained and a differential cell count was performed on all available cells. Due to rapid coagulation and degranulation of cells, preparations were made immediately after sampling. Reference intervals were calculated using a nonparametric approach with 90% confidence intervals of the reference limits. Hemocyte classification was based on the available literature. These results will help establish baseline reference intervals for use in tarantula medicine and will also allow for a more quantitative assessment and thus a better understanding of arachnid health.

Key words: Cameroon red tarantula, hemocyte, hemolymph, Hysterocrates gigas, reference values, theraphosidae

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LITERATURE CITED


SWEATING THE SMALL STUFF: APPLICATION OF SCIENTIFIC MANAGEMENT INCREASES LABORATORY REPRODUCTION IN STAGHORN CORAL (*Acropora cervicornis*)

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Abstract

Propagation of staghorn coral (*Acropora cervicornis*) by sexual reproduction for reseeding of reefs in the Florida Keys is an ongoing conservation effort by Florida Aquarium and the Coral Restoration Foundation involving multiple partners (Conservation of Reef Life: CORL). Gamete bundles collected from offshore nurseries were dispersed and separated into oocytes and sperm using a transfer pipette. Oocytes were incubated in 5-gallon buckets with lemonade concentration sperm, a visual concentration historically used for large-scale field production (actual concentration: 9 × 10^6/ml), or 1 × 10^5/ml, calculated using a hemocytometer. After 2 hr, presumed embryos were collected and cleaned three times before transferring to different types of tanks: a) Kreisel, b) modified Kreisel, c) static tank, or d) *Artemia* cone. After 10 hr, approximately 100 eggs/embryos were counted to determine fertilization rates and embryo development. Higher fertilization rates (64 ± 5.4% versus 47 ± 11.3%) and embryo development rates (32 ± 4.4% versus 22 ± 9.9%) were obtained with lower sperm concentrations. Higher embryo development rates were obtained using the *Artemia* cone (44 ± 6%) versus the Kreisel, modified Kreisel, and static tanks, (21 ± 4.8%, 26 ± 6.3%, 34 ± 7.3%, respectively), even though fertilization rates were similar. Further, embryos from the *Artemia* cone were subjectively assessed as more uniform in shape and healthier. Future studies will investigate whether improved larval settling rates are linked to improved embryo morphology. Application of scientific management and attention to detail can improve reproductive rates in marine invertebrate species in large scale field propagation efforts.

**Key words:** *Acropora cervicornis*, embryo, in vitro fertilization, sperm concentration, staghorn coral

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LACK OF EFFECT OF INJECTABLE ALFAXALONE IN THE MADAGASCAR HISSING COCKROACH (Gromphadorhina portentosa) AND ORANGE-SPOTTED COCKROACH (Blaptica dubia): A PILOT STUDY

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Abstract

Fifteen male Madagascar hissing cockroaches (Gromphadorhina portentosa) and fifteen male orange-spotted cockroaches (Blaptica dubia) were used to determine a safe and efficacious dosage of alfaxalone and to evaluate its use in insects. Alfaxalone dosages of 100, 150, 200, 250, 300, 400, 500, and 750 mg/kg were evaluated in G. portentosa. One animal was used to evaluate each dosage with the exception of the 750 mg/kg trial. The first animal used had some leakage, so an additional G. portentosa was used for that trial. The remaining G. portentosa received no treatments and were used as controls. Alfaxalone dosages of 100, 250, 500, and 750 mg/kg were evaluated in B. dubia. One animal was used to evaluate each dosage and the rest were used as controls. G. portentosa and B. dubia used in the trials were weighed and assessed for a righting response prior to each trial. Alfaxalone was delivered intracoelomically to an animal and loss of movement and righting response were evaluated by direct observation. After 20 min post injection, unanesthetized animals were moved to a holding container to monitor for delayed effects. No signs of anesthesia were observed after 6 hr for G. portentosa and 2 hr for B. dubia. Mortalities included five G. portentosa, three of which were in the control group. The other two received an alfaxalone dosage of 750 mg/kg. It is not known at this time whether those two mortalities were associated with the anesthetic protocol used. There were no mortalities in the B. dubia.

Key words: Alfaxalone, anesthesia, Blaptica dubia, Gromphadorhina portentosa, Madagascar hissing cockroach, orange-spotted cockroach

INTRODUCTION

Invertebrates are playing an increasing role in veterinary medicine as zoos, museums, and private collectors maintain large invertebrate collections. Insects are just one group of invertebrates that are of particular interest due to their diversity, potential to be captivating exhibit animals, and the conservation value of some species. The success of physical restraint in insects for examinations and/or procedures mainly depends on the size and species of insect being handled. In most cases, insects can be manually restrained without risk to animal or handler; however, physical restraint is not always possible. Many species are fast moving, can escape restraint, and be difficult to recover. Another challenge is that some species are fragile and easily damaged with restraint. Therefore, the development of anesthetic protocols is an important step in advancing invertebrate medicine. Historically, hypothermia and CO₂ gas have been used for anesthesia in entomological research. The use of these methods is controversial due to multiple side effects, such as convulsion.
and excitation at induction and high mortality.\textsuperscript{3,7,8} Anesthetic inhalants are a more progressive approach and are commonly used in terrestrial species by placing the animal in an anesthetic chamber through which the agents are pumped. Anesthetic inhalants used include isoflurane, sevoflurane, and halothane.\textsuperscript{3,7,8} Drawbacks to using gas anesthesia include the uncertainty in the amount of drug the animal is receiving and repeated exposure to the inhalant may be needed to keep the animal under anesthesia. The latter can result in release of the inhalant into the environment and unintended exposure to personnel. A safe, reliable and effective injectable anesthetic protocol would allow for full access to the animal without requiring repeated or constant exposure to anesthetic gases.

Alfaxalone (Alfaxan®; 10 mg/ml, Jurox Inc, Kansas City, Missouri 64111 USA) is an intravenous injectable anesthetic licensed for use in cats (Felis catus) and dogs (Canis familiaris) for the induction and maintenance of anesthesia.\textsuperscript{1} Alfaxalone induces anesthesia through activity at the gamma-aminobutyric acid sub-type A receptor (GABAA) by enhancing the effects of GABA, a major inhibitory neurotransmitter in the central nervous system.\textsuperscript{2} Its use has been described in invertebrates such as the Chilean rose tarantula (Grammostola rosea) and blue crab (Callinectes sapidus).\textsuperscript{4,6} To the best of the authors’ knowledge, the efficacy of alfaxalone has not been evaluated in the Madagascar hissing cockroach (Gromphadorhina portentosa) and orange-spotted cockroach (Blaptica dubia).

**MATERIAL AND METHODS**

Fifteen male *G. portentosa* and fifteen male *B. dubia* were provided by the Bug Zoo located in the Plant Sciences Building at Colorado State University. The range of weights for *G. portentosa* was 4-6 g, while *B. dubia* weighed 1 g. The animals were housed by species following the care instructions provided by the Bug Zoo. The *G. portentosa* colony was housed in a 28 × 18 × 16 cm container using cardboard egg cartons as substrate. The *B. dubia* colony was housed in a 21 × 13 × 13 cm container using the same substrate. Both containers were placed inside a 111 × 65 × 75 cm treatment cage (Snyder Manufacturing Company, Centennial, Colorado 80111 USA) with the temperature set to 23.9°C (optimal temperature: 23.9-29.4°C) with 50% humidity (optimal humidity: > 30% without water running down the sides of the cage). The treatment cage malfunctioned the day the animals were housed inside, causing the temperature to decrease to 12.8°C overnight. Climate control using the treatment cage thereafter was discontinued. The colonies were then maintained at room temperature (20.8-22.5°C) with a heating lamp provided for 10-12 hr. Humidity was provided by placing wet paper towels in the containers. The paper towels were changed daily and moistened as needed. The animals were fed an apple or orange slice three times a week, with the uneaten fruit removed the next day. The animals were acclimated for 5 days prior to initiation of the study. Although the approval of the Animal Care and Use Committee is not required by Colorado State University for invertebrates, all efforts were taken to minimize stress and discomfort to the animals used in this study. Animals at the end of the study were returned to the Bug Zoo.

*G. portentosa* and *B. dubia* used in the trials were weighed and assessed for a righting response prior to each trial. Alfaxalone dosages of 100, 150, 200, 250, 300, 400, 500, and 750 mg/kg were evaluated in *G. portentosa*. One animal was used to evaluate each dosage with the exception of the 750 mg/kg trial. The first animal used had some leakage, so an additional *G. portentosa* was
used for that trial. The remaining *G. portentosa* received no treatments and were used as controls. Alfaxalone dosages of 100, 250, 500, and 750 mg/kg were evaluated in *B. dubia*. One animal was used to evaluate each dosage and the rest were used as controls. Alfaxalone was delivered intracoelomically to an animal and then it was observed for loss of movement and righting response. Total immobility, absence of a righting response, and apparent lack of awareness to stimuli indicate full anesthesia in insects. A scoring system was designed to assess anesthesia in *G. portentosa* and *B. dubia*. Scored parameters included tactile stimulation of limbs, antennae, and cerci as well as hissing in the case of *G. portentosa*.

After 20 min post injection, unanesthetized animals were moved to a holding container to monitor for delayed effects by visual evaluation of total immobility. These animals were assessed every 30 min for the duration of the trials: 6 hr for *G. portentosa* and 2 hr for *B. dubia*.

**RESULTS**

No signs of anesthesia were observed in any of the cockroaches at any of the administered dosages. Mortalities included five *G. portentosa*, three of which were in the control group. Of the three in the control group, one died during the acclimation period. The other two died during the *G. portentosa* alfaxalone trial period. The two remaining mortalities received an alfaxalone dosage of 750 mg/kg. They died after returning to the Bug Zoo at the end of the study, which was 1 wk after their respective trial date. These animals were found deceased in their enclosure. No necropsy or histopathology was performed on deceased animals. There were no mortalities in the *B. dubia*.

**DISCUSSION**

This pilot study suggests that alfaxalone is not an effective anesthetic drug for *G. portentosa* and *B. dubia*. Although the function is similar, the pharmacology of the insect GABA receptor differs from that of the vertebrate GABAA receptor. Several steroids that are active on vertebrate GABAA receptors are much less effective on insect GABA receptors. This is the case for pregnane steroids. Alfaxalone is a neuroactive steroid molecule that is a synthetic analogue of progesterone, but it does not bind to sex hormone, glucocorticoid, or mineralocorticoid receptors. The authors suspect alfaxalone’s pharmacologic profile did not allow it to exert an effect on the steroid site on the insect GABA receptor when used at the dosages and route administered.

While the cause of death for the *G. portentosa* mortalities cannot be elucidated, it is likely that the cold shock experienced from the treatment cage malfunction played a role. *G. portentosa*, originally from Madagascar, evolved to be adapted to tropical climates. Spending the night at 12.8°C may have caused excessive stress in some individuals. Since *G. portentosa* in both the treatment and control groups experienced mortalities, it is less likely that the mortalities in the treatment group was solely due to the anesthetic protocol. This is further supported by the lack of mortality in the *B. dubia*. The two *G. portentosa* mortalities in the treatment group did receive 750 mg/kg, but they died 1 wk after their trial date. The cause of death was not identified in this study.

The maximum dosage used in this study was 750 mg/kg, which equated to a maximum fluid volume of 375 µl for *G. portentosa* and 75 µl for *B. dubia*. Higher dosages were not used due to leakage that higher injection volumes would have caused. The licensed dosage range for alfaxalone
is 2.2-9.7 mg/kg for _F. catus_ and 1.5-4.5 mg/kg for _C. familiaris_. This is in stark contrast to the 750 mg/kg used in this study without an effect on cockroaches. This trend of higher dosages of alfaxalone in invertebrates is seen in _G. rosea_ and _C. sapidus_. Alfaxalone administered at 200 mg/kg in _G. rosea_ induced about 10 min of moderate anesthesia. The majority of _G. rosea_ retained muscle tone and sensitivity to stimulation at this dosage. When used in _C. sapidus_, an alfaxalone dosage of 15 mg/kg provided adequate anesthesia for about 11 min, during which none of the animals responded to tactile stimulation. At 100 mg/kg, the length of anesthesia was prolonged to just over 1 hr. The reason for the various dosages in different invertebrate species is unknown at this time, but factors such as drug profile, differences in drug binding sites, metabolism, and elimination may play a role.

The invertebrates are a diverse group of animals and require special anesthetic considerations. In the case of alfaxalone, higher dosages are required for invertebrates compared to mammalian species. Higher dosages translate into higher injection volumes, which is difficult to administer given the size of some invertebrates. This requires a more concentrated formulation of a drug or new drugs/drug combinations. Since the steroid site is weakly effective in insects, future studies should target the other sites on the GABA receptor. One promising site is the benzodiazepine binding site, which shows similarities to the peripheral benzodiazepine sites of vertebrates. Once a safe and efficacious injectable drug is found for insects, the next step is to find a concentration for aerosolized delivery to facilitate ease of administration.

**ACKNOWLEDGMENTS**

The authors would like to thank the Colorado State University Bug Zoo for providing the cockroaches used in this study. They also thank Jurox for its generous donation of one bottle of alfaxalone.

**LITERATURE CITED**


TREATMENT OF GLAUCOMA IN A MALE GORILLA (Gorilla gorilla gorilla) USING TRANSSCLERAL MICROPULSE LASER THERAPY

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Abstract

A 23-yr-old male Western lowland gorilla (Gorilla gorilla gorilla) presented with a 1-wk history of progressive vision impairment. Additional clinical signs at presentation included intermittent profound lethargy and presumed head pain. Initial exam under anesthesia (day 0) revealed bilateral glaucoma (IOP 50-54 mm Hg o.d. and 55-60 mm Hg o.s.). Treatment initially consisted of oral acetazolamide (500 mg p.o. daily, increased to b.i.d.; Heritage Pharm, Eatontown, New Jersey 07724 USA) and ibuprofen (800 mg p.o. daily; Major Pharm, Livonia, Michigan 48150 USA). The animal underwent ciliary body ablation laser surgery on day 14 using a transscleral micropulse cyclophotocoagulation (240 sec/eye, 120 sec ventral, 120 sec dorsal, o.u. at 2,000 mW × 31.3% duty cycle) (Novel micropulse P3 glaucoma device, Iridex, Mountain View, California USA). Post treatment, the animal’s vision was initially worse which was attributed to postoperative uveitis and on day 22 the gorilla developed bilateral hyphema. Neither complication is common in humans treated in the same manner but both improved with treatment including topical dorzolamide/timolol o.u., t.i.d. (Hi-Tech Pharm., Amityville, New York 11701 USA) and neomycin/polymyxin/dexamethasone drops o.u., b.i.d. (Bausch & Lomb, Tampa, Florida 33637 USA). Vision returned to pre-procedure levels by day 49. Recheck of IOP under anesthesia on day 53 revealed resolution of the glaucoma (IOP 13 mm Hg o.s., 9 mm Hg). On day 58 the animal started displaying intermittent episodes of disorientation. Treatment with memantine (5 mg p.o. daily initially, increased to b.i.d. after 5 days; Actavis Pharm., Parsippany, New Jersey 07054 USA) was initiated and the disorientation improved over the subsequent 2 mo. It is unclear if this disorientation was related to the unknown underlying cause of the glaucoma or possibly simply a result of decreased vision subsequent to the glaucoma. Over the following 4 mo, oral and topical medications were weaned with the exception of memantine which is ongoing. Although significant vision loss resulted from the glaucoma, the vision is stable, no signs of pain are present, and the animal is able to function as the dominant male in the social group.

Key words: Glaucoma, Gorilla gorilla gorilla, laser

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The authors thank the Iridex company for donating time and use of their laser. The authors also thank the veterinary and mammal department staff of the Wildlife Conservation Society for the ongoing care of this animal.
CONGENITAL HYPOTHYROIDISM IN A BORNEAN ORANGUTAN (Pongo pygmaeus) AND A SUMATRAN ORANGUTAN (Pongo abelii)

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Abstract

Adult-onset hypothyroidism has previously been reported in a chimpanzee (Pan troglodytes), a Western lowland gorilla (Gorilla gorilla gorilla), and two Bornean orangutans (Pongo pygmaeus).1-5 To the authors’ knowledge, primary congenital hypothyroidism (CH) has not been described in great apes. In humans, CH is most commonly due to thyroid dysgenesis (athyreosis, ectopy, or hypoplasia). The remaining cases are caused by inherited defects in thyroid hormone biosynthesis (dyshormonogenesis).6 The first case is a 9-mo-old male Bornean orangutan presented with delayed development, myxedematous facies, hypotonia, macroglossia, and abdominal distension. Genetic testing, including karyotype, fluorescence in situ hybridization (FISH) analysis, and chromosome microarray analysis (CMA) was utilized to rule out Down syndrome and other inherited disorders with similar clinical signs. Thyroid sonography performed by a board-certified pediatric radiologist showed that there was no discernible thyroid tissue present. Thyroid scintigraphy using Technetium-99m detected only minimal radionuclide uptake in the anatomic region of the thyroid gland consistent with severe hypoplasia. The second case is a 6-wk-old female Sumatran orangutan (Pongo abelii) presented with a poor feeding response, lethargy, impaired thermoregulation, jaundice, and anemia. While commercially available thyroid hormone assays for humans are not validated for use in orangutans, when compared to clinically healthy orangutans of similar age, serum thyroxine (T4) level was significantly decreased and thyroid stimulating hormone (TSH) level was markedly elevated in both cases. Oral supplementation with levothyroxine sodium resulted in noticeable clinical improvement in both cases with near resolution of all clinical signs in the Bornean orangutan within 30 days of initiating treatment.

**Key words:** Congenital, endocrinopathy, hypothyroidism, orangutan, *Pongo abelii*, *Pongo pygmaeus*

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MANAGEMENT OF Clostridium difficile INFECTION IN A COLONY OF BLACK-HANDED SPIDER MONKEYS (Ateles geoffroyi)

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Abstract

Clostridium difficile has historically been recognized in humans as a cause of diarrhea in elderly, hospitalized and immunocompromised individuals.1,2 Over the last decade, it has increasingly become community-acquired and has been identified in young, healthy humans.1,2 It has rarely been identified in nonhuman primates.3 An adult male black-handed spider monkey (Ateles geoffroyi) at a zoo suffered an acute onset of anorexia, depression, and diarrhea that failed to respond to antibiotic and fluid therapy, resulting in death within 24 hr of the first observed clinical signs. A diagnosis of fibrinonecrotic enterocolitis was made on necropsy and C. difficile was isolated from the intestines. The four remaining spider monkeys in the colony subsequently developed moderate to severe watery diarrhea, depression and anorexia. Serial samples collected over 8 wk confirmed toxigenic C. difficile in all four animals. Initial treatment with metronidazole was discontinued when animals refused to take the antibiotic orally. Treatment with oral vancomycin resolved clinical signs until antibiotics were discontinued. Subsequent treatment with endoscopic fecal microbiota transplantation using material prepared from feces from spider monkeys at another institution, resulted in engraftment of the donor’s fecal microbiomes in all four monkeys, resolution of diarrhea, and absence of C. difficile and its toxins from subsequent stool studies.1 Microbial samples from the index case and three of the four surviving animals were subtyped by the state department of health and were found to be different than the community NAP types in their database, but 80% similar to each other based on PGFE subtyping.

Key words: Ateles geoffroyi, black-handed spider monkey, Clostridium difficile, fecal microbiome, microbiota transplantation

LITERATURE CITED


REVIEW OF REPRODUCTION IN CAPTIVE WESTERN GORILLAS (Gorilla gorilla), 1996-2016

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Abstract

To gain an understanding of the prevalence of veterinary intervention during parturition and neonatal care of gorillas (Gorilla gorilla) in captivity, a survey was sent to all of the zoological institutions associated with the Association of Zoos and Aquariums’ Gorilla Species Survival Plan® (SSP). Of the 53 institutions contacted, 50 responded to the survey (94.3%), with a total of 192 births reported between 1996-2016. Of these 192 reported births, there were five incidences of prolonged labor (longer than 6 hr; 2.6%), six cases of cesarean section (3.1%), and one incidence of veterinary intervention that did not involve a cesarean section. According to the survey responses, 51 infants out of 186 live births (27.4%; 95% confidence interval 20.8-33.2%) were hand-reared or required veterinary intervention lasting longer than 24 hr. Out of 33 attempts to reintroduce neonates to the family group, 25 were successful (78.8%, 95% CI 62.0-89.6%); of 17 attempts to introduce neonates to surrogates, 15 were successful (88.2%, 95% CI 64.1-98.0%). Nine introduction attempts (18.3%, 95% CI 9.8-31.2%) were deemed failures, with one (2.0%, 95% CI < 0.001-11.7%) resulting in the death of the infant. Compared to data from a similar survey conducted in 1981, the percentage of hand-reared gorillas has decreased from 64% to 27.4% (P < 0.0001); however, there is a high success rate of reintroduction after intervention during birth (cesarean section or other) to either the natal troop or a surrogate. This indicates that intervention during parturition could be lower risk than previously thought, and could be considered as an alternative to natural birth in high-risk pregnancies.

Key words: Cesarean section, Gorilla gorilla, hand-rearing, infant mortality, reproduction, Western gorilla

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Zoological Park, Toronto Zoo, Utah’s Hogle Zoo, Woodland Park Zoological Gardens, Zoo Atlanta, Zoo Miami and Zoo New England for their participation in this study.

LITERATURE CITED

CALLITRICHID PREVENTIVE MEDICINE PROTOCOLS AT THE BRONX ZOO: A CASE EXAMPLE OF THE RE-EVAUATION PROCESS

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Abstract

Preventive medicine protocols (PMP) are important tools for keeping zoological animals healthy, but to be effective they need to be regularly reviewed and updated. Bronx Zoo callitrichid PMP included yearly fecal ova and parasite (O&P) evaluation and scheduled anthelmintic treatments. Protocols were last adjusted in 2009 to include monthly anthelmintics in response to increased cases of Gongylonema. O&P (n = 1360), enteric culture (n = 417), and necropsy (n = 141) results from January 2000 to July 2016 were reviewed for all captive callitrichids (16 species) at the Bronx Zoo. O&P tests were infrequently positive (4-9%), revealing incidental parasites in nearly all cases. Necropsy results identified 10 cases of pancreatic nematodiasis—presumptive Trichospirura leptosoma—seven of which (5%) were significant, and 12 cases of Gongylonema spp. infection. All of these nematodiasis cases occurred prior to 2013. Fecal O&P examinations failed to detect the presence of either of these parasites. Antemortem diagnostic enteric cultures were frequently positive (28%) for clinically relevant organisms. The same pathogens were occasionally cultured at necropsy, but only Klebsiella sp. was consistently found both ante- and postmortem in the same animals (n = 9).

Analysis of these results indicated that 1) O&P screening was insufficient to detect significant parasitic disease, but necropsy results were very useful; 2) enteric cultures were often helpful in identifying pathogens; and 3) necropsy results were by far the most useful tool to help adjust PMP. As a result of this analysis annual O&P screening for healthy individuals has been discontinued; the annual screening was clearly not sensitive enough to be helpful in making treatment decisions. An alternative approach considered was to increase the frequency of fecal monitoring of healthy individuals, but it was decided that necropsy surveillance and continued screening of sick and shipping individuals would be sufficient to identify parasitic infections that require a change in the preventive medicine treatments. In addition, the aggressive deworming protocol of monthly anthelmintic treatments is believed to have disrupted the life cycle of these parasites in callitrichids at the Bronx Zoo and thus routine treatments have been decreased to once yearly ivermectin and once yearly fenbendazole. The authors also have adjusted the approach to diarrhea cases to perform a culture earlier in the clinical course. This presentation serves as an example of how PMPs should be periodically re-evaluated in order to tailor PMP to both the individual institution and to changes in disease prevalence over time.

Key words: Callitrichidae, parasitology, preventive medicine
AVOCADO (Persea americana) TOXICITY IN CAPTIVE AYE-AYE (Daubentonia madagascariensis)

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Abstract

Avocado leaves, pits, skin, and possibly fruit contain a toxin called persin that has been associated with acute myocardial degeneration following ingestion in a variety of mammalian and avian species.1-4 Susceptibility to persin appears to be highly variable between species and there appear to be factors related both to the avocado and the animal that affect the susceptibility to toxicity. Ingestion of persin targets heart and lactating mammary gland and results in clinical symptoms that vary with species but include a sudden onset of weakness, which may progress rapidly to death.4 Five of 13 aye-aye (Daubentonia madagascariensis) housed at the Duke Lemur Center became ill within a few hours of each other and within the next 15 hr four of the five had died. All five of the ill aye-ayes developed pericardial effusion and had been fed avocado fruits (Persea americana) the day prior to death from acute myocardial degeneration. The four animals that died all had stomach contents containing persin. A review of necropsy results from unexplained acute aye-aye deaths over the past 20 yr uncovered an additional five cases at four other institutions with necropsy and histopathology findings strongly suggestive of avocado toxicity. The unusual factor in the present case was a history of long standing avocado ingestion in the colony without morbidity/mortality, suggesting a co-factor. The aye-aye is the first reported primate avocado toxicity. The authors speculate that catecholamine effects of stress on the heart may turn out to be the as-yet unidentified modifier of toxicity.

Key words: Avocado, aye-aye, Daubentonia madagascariensis, pericardial effusion, persin, toxicity

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LITERATURE CITED


APPLICATION OF COMMERCIAL Aspergillus WESTERN BLOT IGG® KIT AND ELISA ASSAY FOR THE DIAGNOSIS OF ASPERGILLOSIS IN COMMON BOTTLENOSE DOLPHINS (Tursiops truncatus)

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Abstract

The definitive diagnosis of aspergillosis is difficult in animals.1,2 Thus, new laboratory tools are necessary to overcome current diagnostic limitations of low specificity and lack of standardization. This study of common bottlenose dolphins (Tursiops truncatus), evaluated the diagnostic performance of a new commercial immunoblot kit, the Aspergillus Western blot IgG® (LDBio Diagnostics, Lyon, France), that had been initially developed for the serologic diagnosis of chronic aspergillosis in humans.1 Intensity of four Aspergillus-specific bands were quantified using the kit. Results were compared with those obtained by a novel ELISA test, within an observation cohort of dolphins with proven or probable diagnosis of aspergillosis (n = 32) and negative controls (n = 55). Overall, the diagnostic performance of the commercial immunoblot and ELISA were strongly correlated (P < 0.0001). The immunoblot showed lower sensitivity (65.6% versus 90.6%), but higher specificity (92.7% versus 69.1%), with no cross-reaction with miscellaneous non-Aspergillus fungal infections, while the ELISA appeared to cross-react with non-Aspergillus spp. When assessing their use in a validation cohort of 21 dolphins under human care and 32 free-ranging dolphins, the commercial immunoblot kit and the ELISA assay enabled positive diagnosis before mycological cultures in 42.9% and 33.3% of dolphins tested for suspicion of aspergillosis, respectively. There also was significant impact of antifungal treatment on the results of the two tests (P < 0.05). In all, these new serologic methods show promise in aiding in the diagnosis of aspergillosis in dolphins, and illustrate the opportunity to adapt reagents directed for human diagnostics to detect similar changes in other animals.

Key words: aspergillosis, Aspergillus spp., dolphins, immunoblot, ELISA, serology, Tursiops truncatus
ACKNOWLEDGMENTS

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LITERATURE CITED


RETROSPECTIVE REVIEW OF MORBIDITY AND MORTALITY IN FROGFISH (ANTENNARIIDAE), 2002-2015

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Abstract

Frogfish (Antennariidae) are a popular display animal in aquariums due to their unique anatomy and cryptic coloration. A lack of knowledge exists concerning common diseases encountered in frogfish. A retrospective review of clinical records, gross pathology, and histopathology was conducted on captive frogfish specimens at the Shedd Aquarium from 2002 through 2015 to identify causes of morbidity and mortality. The study population consisted of 26 individuals (three alive at time of data collection) representing six species of frogfish (Antennarius hispidus, A. maculatus, A. commerson, A. biocellatus, A. avelonis, and A. bermudensis). Gill biopsies, tissues, and whole body specimens were submitted for necropsy and histopathology. Endocardial hypertrophy, melanomacrophage hyperplasia, and hepatic lipid deposition were found to be common reactions to illness in the frogfish. Review of histopathology records revealed that sepsis was the most common cause of mortality at 47.8% (11 of 23 cases). The two most common causes of sepsis were fungus (55%, 6 of 11 cases) and bacteria (45%, 5 of 11 cases). Exophiala species were the most common fungal infection, whereas Vibrio species were the most common bacterial etiology. Common parasites included the pathogenic Cryptocaryon irritans and incidental Myxosporidia and Microsporidia species. The findings of this study will allow for improved care and management of frogfish in an aquarium setting.

Key words: Antennariidae, Cryptocaryon, Exophiala, frogfish, Vibrio

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Authors would like to thank The University of Illinois Zoological Pathology Program. Thank you to the Shedd Aquarium Animal Health and Fishes Departments with special thanks to the veterinary technicians and aquarists.
COMPARISON OF A SMARTPHONE-BASED ELECTROCARDIOGRAM DEVICE WITH A STANDARD SIX-LEAD ELECTROCARDIOGRAM IN THE ATLANTIC BOTTLENOSE DOLPHIN (*Tursiops truncatus*)

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Abstract

A smartphone-based bipolar, single-lead electrocardiogram (ECG) device⁴ is capable of recording electrocardiograms (ECG) with an integrated smartphone⁵ application.⁶ To determine the utility of this device, a comparison was conducted of phone-based ECGs (pECG) to standard six-lead ECGs⁷ (sECG) in four female Atlantic bottlenose dolphins (*Tursiops truncatus*) at the National Aquarium. Study animals were trained to haulout onto a dry deck in ventral recumbency and to allow simultaneous 30-sec ECG acquisition using the two devices. The pECG device was held against the thoracic wall caudal to the left axilla. The standard six-lead ECGs were recorded in the frontal plane as previously described.⁸ Instantaneous heart rates were obtained from identical QRS complexes on both ECGs. Three board certified cardiologists independently evaluated the rhythm and the polarity of the QRS depolarization for each recording. The results were compared between observers. The mean heart rate with the pECG was 80 bpm, (range 62-92 bpm) and 80 bpm (range 60-90 bpm) with the sECG. All four dolphins displayed sinus arrhythmia and one animal had occasional atrial premature contractions. Rhythm diagnosis and QRS polarity were identical for the pECG device and sECG. Dolphin vocalization created artifacts on the pECG that were not present on the sECG requiring acquisition to occur without vocalization. The pECG appears to be accurate and useful to acquire a diagnostic ECG in Atlantic bottlenose dolphins. This system is inexpensive and portable making it valuable for health examinations, transport monitoring, and stranding responses.

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Key words: AliveCor, bottlenose dolphin, electrocardiogram, smartphone, *Tursiops truncatus*

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LITERATURE CITED


DETECTION OF HERPESVIRUS IN WILD POPULATIONS OF SOUTH AMERICAN SEA LIONS (*Otaria byronia*) AND PERUVIAN FUR SEALS (*Arctocephalus australis*) IN PERU

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Abstract

Four gammaherpesviruses have been described in otariids, three of which have been primarily found in California sea lions (*Zalophus californianus*).1-4 Otarine herpesvirus-1 (OtHV-1) is closely associated with a high incidence of urogenital carcinoma in CSL.1,4 A single Peruvian fur seal (*Arctocephalus australis*) with urogenital carcinoma has been observed, with OtHV-1 likely contracted in managed care, but little is known about the herpesviruses present in free-ranging populations of South American otariids.3 While herpesviruses tend to be relatively host specific, host jumping has been reported in closely related species.3 OtHV-1 in CSL and otarine herpesvirus-4 in Northern fur seals (*Callorhinus ursinus*) appear to be very closely related.2 This study tested 67 genital swabs from a longitudinal study on South American pinnipeds from 2011, 2014, and 2015 using a consensus PCR. There was an overall prevalence of 21% (*n* = 14). Sequencing revealed 99% homology to OtHV-1. Positive samples were observed mostly in Peruvian fur seals (*n* = 13; 29% species prevalence), with a single sample from a South American sea lion (*Otaria byronia*; 5% species prevalence). Prevalence was not significantly different between 2011 (24%), 2014 (17%), and 2015 (18%). No animals showed any clinical signs of disease during their examinations. This study demonstrates, for the first time, the presence of herpesvirus in apparently healthy, free-ranging Peruvian fur seals and South American sea lions.

Key words: *Arctocephalus australis*, gammaherpesvirus, *Otaria byronia*, otarine herpesvirus, Peruvian fur seal, South American sea lion

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LITERATURE CITED


RETROSPECTIVE ANALYSIS OF PERIANESTHETIC MORTALITY RISK FACTORS IN CALIFORNIA SEA LIONS (Zalophus californianus) UNDERGOING REHABILITATION

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Abstract

Anesthetic techniques in marine mammals have been refined in recent years, but still carry a greater risk of associated mortality in comparison to other species.3,4 Known complications in otariids include prolonged recoveries, thermoregulatory compromise, and death.1,2 In an effort to evaluate and understand the risk factors in California sea lions (CSL) (Zalophus californianus) undergoing treatment at The Marine Mammal Center, and to reduce mortality during anesthetic procedures, a retrospective case-control study was undertaken. Medical and anesthetic records for all CSL admitted between 2013 and 2016 were evaluated. A total of 809 anesthetic procedures were performed during the study period. Perianesthetic deaths were defined as having occurred during the induction, maintenance, or recovery periods, or within 48 hr of the anesthetic procedure (n = 58). Animals that recovered and were alive 48 hr post procedure were classified as controls (n = 647). Risk factors including sex, age class, body weight, health status, days since admission, duration of anesthesia, premedication/induction agent(s), maintenance agent, endotracheal intubation, antagonist administration, and prior anesthesia were evaluated. Overall, there was a 2.8% (n = 20) mortality rate during anesthetic induction and maintenance; this increased to 6.5% and 8.2% at 24 and 48 hr following recovery, respectively. Odds ratio calculations revealed that significant risk factors included age, health status, days since admission, and duration of anesthesia. Evaluating the data garnered from ongoing anesthetic efforts in stranded CSL will continue to expand the knowledge regarding anesthetic risks and inform options for decreasing perianesthetic mortality.

Key words: Anesthesia, California sea lions, perianesthetic death, retrospective, Zalophus californianus

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LITERATURE CITED


EVALUATION OF SERUM PROLACTIN AS A MEANS OF PREGNANCY DIAGNOSIS IN FLORIDA MANATEES (Trichechus manatus latirostris)

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Abstract

Identification of reproductive status in the adult female Florida manatee (Trichechus manatus latirostris) can be challenging, as physical exam and diagnostic imaging are unrewarding until late gestation.2 Serum progesterone, the preferred analyte for pregnancy diagnosis in manatees, requires at least two progesterone measurements to confirm pregnancy status.2 Serum prolactin has been described as a single-sample pregnancy diagnostic for Asian (Elephas maximus) and African (Loxodonta africana) elephants, which are close relatives to manatees.1 In the present study, serum samples were analyzed from a total of 41 rescued female manatees admitted to Tampa’s Lowry Park Zoo between the years 2000 and 2016. Each sample was classified based on reproductive status as nonpregnant/nonlactating adult (n = 9), pregnant (n = 17), lactating (n = 22), or subadult (n = 7). Reproductive status was determined based on one of the following: delivery of a calf or stillborn fetus during rehabilitation, presence of a calf at rescue, presence of fetus at necropsy, visualization of fetus on ultrasound, or by confirmation of elevated progesterone levels. Statistical analysis was performed using the independent samples t-test to compare mean prolactin levels among the reproductive classification groups. Prolactin levels were significantly higher in pregnant females (17.93 ± 24.85 ng/ml) than nonpregnant adult and subadult females (2.35 ± 1.08 ng/ml; P = 0.02). The results of this study show that serum prolactin is significantly higher in pregnant animals, supporting the potential use of this assay for pregnancy diagnosis in the Florida manatee.

Key words: Florida manatee, pregnancy, prolactin, Trichechus manatus latirostris

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LITERATURE CITED


INFLUENCE OF IODINE SUPPLEMENTATION ON SERUM T₃ AND T₄ CONCENTRATIONS IN WHITE-SPOTTED BAMBOO SHARKS (*Chiloscyllium plagiosum*)

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Abstract

Goiter, a pathologic enlargement of the thyroid gland, is a significant disease of captive sharks, and causes morbidity (e.g., goiter rupture, anorexia) and mortality.¹ A longstanding hypothesis with respect to the underlying cause of goiter in sharks maintains that goiter results from insufficient iodine in the diet or tank water; however, results of recent studies demonstrated that other factors, such as nitrates, may contribute to the development of goiter in sharks.² The role of iodine in normal shark thyroid physiology is currently uncertain, and definitive recommendations regarding iodine supplementation (e.g., oral versus tank water supplementation, accurate supplement quantity) cannot be made at this time. In order to determine the interaction between iodine supplementation and serum thyroid hormone concentrations, healthy adult white-spotted bamboo sharks (*Chiloscyllium plagiosum*) (n = 28) were enrolled in this study. Four groups of sharks (n = 7 per group) received one of the following iodine supplementation protocols: 1) no iodine supplementation; 2) oral iodine supplementation; 3) water iodine supplementation; or 4) both oral and water iodine supplementation. Results suggested that serum T₄ concentrations were more consistent in sharks that received oral and water supplementation. In addition, serum T₃ concentrations were significantly decreased in sharks receiving oral supplementation. Based on these results, it appears iodine supplementation alters the thyroid hormone concentrations of captive white-spotted bamboo sharks, and that both oral and water supplementation may play a role in appropriate iodine supplementation.

**Key words:** *Chiloscyllium plagiosum*, iodine, supplementation, thyroid hormone

LITERATURE CITED


SIGNIFICANCE OF SYMMETRIC DIMETHYLARGININE (SDMA) IN EVALUATING RENAL INSUFFICIENCY IN REHABILITATED WILD FLORIDA MANATEES (Trichechus manatus latirostris) AND REFERENCE VALUES IN TWO WILD MANATEE POPULATIONS

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Abstract

Few reports of renal pathology in Florida manatees (Trichechus manatus latirostris) exist in the veterinary literature, yet renal compromise is a significant complication when rehabilitating debilitated manatees.3,4 Animals are frequently dehydrated and may have sustained direct trauma to the kidneys via boat strikes or ischemic events. Clinicians are limited to analysis of blood urea nitrogen, creatinine levels, and urinalysis (if urine can be obtained) to determine the extent of renal insufficiency, which may not accurately reflect renal function as it does in other species.5 This poses a diagnostic challenge for clinicians in discerning how critical renal compromise is to the animal’s overall health and prognosis.

Symmetric dimethylarginine (SDMA) is an accurate measure of glomerular filtration rate in humans, cats, and dogs, and is proven to be a reliable biomarker for early detection and monitoring of chronic kidney disease in those species.1,2,6 Retrospective analysis of SDMA values in 10 wild Florida manatees with known renal disease were shown to be statistically significantly elevated compared to six manatees with no reported renal lesions on histopathology. Serum SDMA values from manatees in Crystal River (February 2013 to February 2017) and Brevard County (January 2009 and December 2014) were comparable to those established in small animal medicine, with Crystal River populations at 12.23 ± 3.13 µg/dl and Brevard County populations at 10.79 ± 3.21 µg/dl.

These data support SDMA as clinically useful in evaluating renal function in manatees. Values obtained from wild populations appear to be comparable to established reference ranges in dogs and cats.6

Key words: Florida manatee, renal disease, SDMA reference ranges, symmetric dimethylarginine, Trichechus manatus latirostris

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LITERATURE CITED


EMERGING DISEASES IN REPTILES AND HOW WE MAY BE CHANGING OUR DEFINITION OF HEALTH

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Abstract

Deteriorating wildlife health threatens the sustainability and success of conservation efforts. Techniques that characterize wellness in wildlife utilize specific biomedical diagnostics. Hematologic, plasma biochemical, and pathogen prevalence data have been utilized as a means of determining the wellness of free-ranging reptile populations, but for the most part have not been critically evaluated. Two infectious diseases that have been proposed as a threat to biodiversity and affect free-ranging and captive reptiles include ranavirus and *Ophidiomyces* (snake fungal disease or SFD). Antemortem diagnostic tests for these pathogens revolve around molecular detection, but the relationship of the pathogen to the host response, as determined by clinical pathology, is unknown. This study investigated both host and pathogen factors that help to shape the health of turtles and snakes in the face of these diseases. It is clear that a multi-modal approach to reptile wellness is required to better characterize these diseases, and reliance on mammalian diagnostic approaches requires modification to be useful in these species. A review of clinical pathology, protein electrophoresis, disease pathogenesis, and treatment modalities will be reviewed for ranavirus and SFD.

Key words: Chelonian, hematology, *Ophidiomyces*, ranavirus, reptile, Serpentes
NOVEL Paranannizziopsis SPECIES IN A WAGLER’S VIPER (Tropidolaemus wagleri) AND TENTACLED SNAKE (Erpeton tentaculatum) IN A ZOOLOGICAL COLLECTION

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Abstract

High mortality was observed in a group of juvenile Wagler’s vipers (WV; Tropidolaemus wagleri) with the consistent finding of widespread retained shed. Skin biopsies were obtained from one viper and revealed a novel Paranannizziopsis species based on phenotypic and molecular tools. Histologic examination revealed hyperplastic, hyperkeratotic, and crusting epidermitis with intralesional fungi. These WV are housed in a room with tentacled snakes (TS; Erpeton tentaculatum) where the terrestrial WV are housed singly in 30.5 × 30.5 × 30.5 cm glass terraria with an average temperature of 25.2°C and relative humidity of 63.2% while TS are housed as a pair in a 56.8-L fully aquatic tank with average water temperature of 25.2°C. The TS in this collection have a history of intermittent skin lesions since 2011. Skin biopsies collected from one TS approximately 2 mo after biopsies collected from the WV revealed a Paranannizziopsis isolate genetically identical to that found in the WV and a similar histologic appearance. Susceptibility testing indicated that terbinafine and voriconazole had potent activity against these isolates. Sequencing confirmed a new species, which was named Paranannizziopsis tardicrebrescens (Mycobank accession 818644), characterized by slow growth on all media, growth at 35°C, hydrolysis of milk solids on bromcresol purple-milk solids-glucose agar, and lacking ascomatal initials and undulate hyphal branches. The route of transmission is unknown, but this fungus is suspected to be present in the environment and transmissible by fomites. Further investigation is needed to understand the natural history of this fungal species.

Key words: Erpeton tentaculatum, fungus, Paranannizziopsis, tentacled snake, Tropidolaemus wagleri, Wagler’s viper

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The authors would like to thank Dr. Jean Paré for providing expert guidance on Paranannizziopsis fungi during the initial investigation of these cases and Fort Worth Zoo’s veterinary technicians and ectotherm department staff for assistance during procedures, administering treatments, and routine care of these animals.
DIAGNOSTIC PERFORMANCE OF READILY AVAILABLE ANALYTES IN THE DIAGNOSIS OF INFLAMMATION IN GOPHER TORTOISES (Gopherus polyphemus)

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Abstract

Hematology can be a useful diagnostic tool for evaluating systemic health in many species and can provide valuable information into the presence of underlying inflammatory disease; however, reptilian hematology is often unreliable since the reptilian patient may exhibit a leukocytosis, leukopenia, or normal leukogram during times of infection and/or inflammation.⁵,⁶ Thus, there exists a need to develop more reliable methods for diagnosing and monitoring inflammation in reptiles. Several readily available diagnostic tests such as erythrocyte sedimentation rate (ESR), lactate, fibrinogen, and plasma protein electrophoresis (EPH) can provide insight into inflammatory disease in a multitude of species.¹-⁴ These tests are nonspecific in that they detect changes caused by inflammation of any etiology, but provide useful information about the presence of systemic inflammation. To the author’s knowledge, none of these analytes have been extensively evaluated in terrestrial chelonians. The objective of this study was to investigate the diagnostic performance of readily available inflammatory markers in the gopher tortoise (Gopherus polyphemus). In total, n = 24 healthy and n = 38 sick animals met the inclusion criteria. Reference ranges for healthy tortoises were established for ESR, lactate, fibrinogen by heat precipitation, EPH, and hematology. Receiver operating characteristic analysis revealed that the highest performing diagnostic tests were ESR (AUC = 0.812; 95% CI = 0.693-0.900), absolute heterophils (AUC = 0.771; 95% CI = 0.646-0.869), WBC estimate (AUC = 0.767; 95% CI = 0.642-0.866), and lactate (AUC = 0.766; 95% CI = 0.641-0.864). These results provide additional tools for the diagnosis and monitoring of inflammatory disease in gopher tortoises. Clinicians may consider adding ESR and lactate to the minimum database for this species.

Key words: Erythrocyte sedimentation rate, gopher tortoise, Gopherus polyphemus, hematology, inflammation, reptile

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LITERATURE CITED


TREATMENT OF CHYTRIDIOMYCOSIS WITH F10 VETERINARY DISINFECTANT

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Abstract

One of the most important drivers of amphibian declines and extinction is the infectious disease chytridiomycosis, which is caused by the fungal pathogen Batrachochytrium dendrobatidis (Bd). In vitro Bd is susceptible to a range of disinfectants, but not all have been tested in animals. Some disinfectants have been proven effective, but have harmful side-effects to animals or the surrounding environment. This study tested the efficacy of F10SC Veterinary Disinfectant to treat Bd in experimentally infected guttural toads (Sclerophrys gutturalis), Power’s toad (S. poweri) and the Phofung River frog (Amietia hymenopus). The minimum inhibitory concentration for F10SC in vitro Bd ranged between 1:7000 for 5 min contact time and 1:10000 for 10 min contact time. Based on the survival data of test animals the no-observed-effect concentration for 15-min contact time was estimated to be 1:2000 dilution for juveniles, and 1:10000 for tadpoles. In S. gutturalis juveniles an 86% infection clearance rate was achieved after five 15-min doses of 1:3000 dilution. A 100% clearance was achieved in A. hymenopus tadpoles after seven 15-min doses of 1:10000 dilution, and after nine doses of the same treatment in S. poweri tadpoles. F10SC shows great promise as a treatment protocol for chytridiomycosis which is nontoxic to tadpoles and post-metamorphic individuals, and is effective against Bd during short contact times, but further testing on different species of amphibians is advised.

Key words: Batrachochytrium dendrobatidis, chytrid fungus, contact time, minimum inhibitory concentration, veterinary disinfectant

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LITERATURE CITED


RETROSPECTIVE EVALUATION OF MYCOBACTERIOSIS IN THE HOUSTON TOAD (*Anaxyrus houstonensis*): CLINICAL PRESENTATIONS, PATHOLOGIC FINDINGS, AND PREVENTION

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Abstract

The Houston toad (*Anaxyrus houstonensis*) is an endangered amphibian native to east-central Texas, USA.2 The Houston Zoo maintains a colony of the species for captive assurance and breeding. Mycobacterial disease has been a significant cause of morbidity and mortality over the past 7 yr. A retrospective study of mycobacteria cases from January, 2009 through July, 2016 was conducted to characterize the most common findings on clinical presentation, the success of antemortem diagnostics, and the most common pathologic findings. One hundred ninety-nine cases were identified. The most common presentations were abnormal posturing or ambulation, joint or toe swelling, and cutaneous swelling, ulcer, or erythema. Histopathology showed that the musculoskeletal and visceral body systems were most commonly affected, with granulomatous osteomyelitis and granulomatous pneumonia most common, respectively. Of the cases that had both cytology and histopathology performed, 67% of cases were positive on both tests, suggesting that cytology is a moderately good antemortem test for mycobacterial disease, but has limitations. Several cases that were positive for acid-fast bacilli on cytology failed demonstrate these organisms on histopathology. This may be due to the strong acids used in the tissue decalcification process,1 and alternative methods of tissue preparation are recommended if trying to detect mycobacterial species.

Key words: *Anaxyrus houstonensis*, atypical mycobacteria, granulomatous osteomyelitis, granulomatous pneumonia, Houston toad, mycobacteriosis

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The authors would like to thank the Houston Toad keepers and the technical staff at the Houston Zoo for their hard work and relentless dedication to this project.

LITERATURE CITED


WHIP-LIKE HETEROPHIL PROJECTIONS IN REPTILES: ART, ARTIFACT, OR SUPPORTIVE OF INFLAMMATION?

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Abstract

Heterophil morphologic changes in reptiles are well characterized regarding their clinical significance, including toxicity, left shifting, artifacts (e.g., from storage or drying), and presence of infectious agents.2 An unusual heterophil morphology was observed in 182 blood films representing 11 reptile species (150 chelonians, 31 snakes, 1 lizard) from 8 zoological institutions. As documented in one of these cases,1 this morphology was characterized by prominent, variably thick, curvilinear, whip-like cytoplasmic projections, which were the same color as heterophil granules. They were present in various proportions of total heterophils with a length of approximately one to three times the heterophil diameter. Most heterophils exhibited one long projection, but some cells had two or more projections trailing into opposite directions. The objective of this study was to evaluate blood films with heterophil projections for evidence of inflammation in the leukogram, and review the clinical history in order to determine the potential clinical significance of this morphologic change. Thirty-nine chelonians with repeat blood films exhibited heterophil projections over time during treatment. Artifacts from sample handling or blood film preparation techniques were excluded as a cause of the projections. The majority of cases (152/182) had concurrent heterophilia, heterophil left-shifting, and/or toxicity, and clinical history of conditions with underlying inflammatory disease; however, the remaining 30 blood film sets were from apparently healthy snakes. Although their cause and significance is unknown, considerations for heterophil projections include an artifact, activation of cells in vitro, or a cellular response to microbes or products of systemic inflammation in vivo.

Key words: Artifact, heterophil, inflammation, reptile

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LITERATURE CITED

PERIPHERAL NERVE SHEATH TUMORS IN A CAPTIVE ASSURANCE COLONY OF HOUSTON TOADS (Anaxyrus houstonensis)

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Abstract

Between August 2013 and July 2016, multiple raised, pigmented skin tumors were noted on the feet of 10 Houston toads (Anaxyrus houstonensis) from the same wild source genetic lineage. Identical masses were also noted on the faces of four toads with a different lineage, but similar county of origin.

Toads behaved normally despite having small to large masses on their feet, often having more than one foot affected. Average age of onset of tumors was 3.8 yr in the foot tumors and 6 yr in the face tumors. Tumors were slow growing and recurred within an average of 15 mo after surgical excision.

Masses were submitted for histopathology and followup electron microscopy, and were diagnosed as multiple tumors of peripheral nerve sheath cells (PNST). These PNSTs share features with neurofibromatosis in people and bovine, with neurofibromatosis type 1 (NF-1) in people caused by mutation of the gene on chromosome 17.

This captive assurance colony of approximately 500 toads is bred yearly for release of offspring to wild habitat, a critical need to bolster decimated wild populations. In fact, recent breeding efforts by the Houston Zoo are recognized as having a major part in preventing extinction of this highly endangered toad, with signs of recovery on the horizon. These tumors posed a unique challenge for this colony and on veterinary decisions regarding diagnosis, management and clearance for breeding, with the possibility of viral involvement and genetic mutation causing the most concern. Unique diagnostics and partnership with human NF-1 specialists were developed.

Key words: Amphibian, Anaxyrus houstonensis, foot, Houston toad, neoplasia, peripheral nerve sheath tumor
HYPERVISCOSITY-LIKE SYNDROME IN REPTILES

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Abstract

Hyperviscosity syndrome (HS) in people is caused by many conditions, ranging from inflammation to cyanosis to plasma cell dyscrasias. It most commonly occurs due to elevations in gammaglobulins, either monoclonal in malignant disease, or polyclonal due to inflammatory conditions such as rheumatism. Clinical signs of HS in people include altered mentation, visual impairment, and bleeding. Hyperviscosity in domestic animals is usually a paraneoplastic syndrome, and has been reported with erythrocytosis and hypergammaglobulinemia secondary to hematopoietic neoplasia. Clinical signs in domestic animals can include erythematous mucus membranes, and neurologic and cardiovascular signs. Hyperviscosity-like syndrome (HLS) in reptiles was first described in Kirtland’s snakes (Clonophis kirtlandii) with proliferative thyroid disease. In this Northwest ZooPath archival study, HLS was diagnosed in snakes and fewer lizards from a wide variety of taxonomic families. Females were more commonly diagnosed with HLS than males, and animals ranged from juvenile to 17 yr old. The most common clinical presentation was acute death without premonitory signs. Blood work was only available in a few cases, which had hyperglobulinemia. Common gross lesions included coelomic effusion, pulmonary edema, and hemorrhage. Histologically, animals consistently had deeply amphophilic fluid in the vasculature and interstitium (proteinaceous edema) in various organs, most commonly the lungs, heart, liver, and kidney. In the majority of cases, HLS was the most substantial finding and considered the most likely cause of death. A minority of cases had substantial concurrent disease, including gout, bacterial infection, neoplasia, and yolk coelomitis. Possible predisposing factors to HLS include hypothermia, sepsis, and endocrine abnormalities.

Key words: Hyperglobulinemia, hyperviscosity syndrome, mortality, pathology, reptiles

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LITERATURE CITED


ADDRESSING THE CHALLENGE OF DO-IT-YOURSELF (DIY) SEMEN BANKING IN WILD FELIDS

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Abstract

Semen collection and cryopreservation are becoming increasingly important for conserving the genetic diversity of declining wild felid populations. Traditionally, semen is collected from anesthetized cats using electroejaculation and then processed through multiple steps prior to straw freezing over liquid nitrogen vapor, requiring specialized equipment and technical skills. Recently, an alternative simplified approach to semen banking has been developed, combining semen collection via urethral catheterization and semen freezing via vitrification.1,2 Although effective in domestic cats, this cath-vit approach has not been tested in wild felids. In this study, objectives were to 1) compare the effectiveness of urethral catheterization/semen vitrification versus electroejaculation/straw freezing in ocelots (Leopardus pardalis), and 2) assess the capacity of zoo veterinarians to opportunistically apply this cath-vit approach for do-it-yourself (DIY) semen banking of their resident felids. For objective 1, urethral catheterization was performed on seven ocelots anesthetized with a combination of ketamine-medetomidine. An aliquot of recovered raw semen was diluted in vitrification medium, held for 5 min and pelleted by direct pipetting into liquid nitrogen. Remaining semen was combined with electroejaculated samples and processed via standard straw freezing methods. Post-thaw analysis included assessment of sperm motility and acrosome status over time and in vitro fertilization (IVF) success following insemination of in vitro matured domestic cat oocytes. In ocelots, four of seven catheterization attempts yielded high quality semen (mean + SE; 63.3 + 47.1 × 10⁶ sperm, 80 + 4% motility) with additional semen (90.5 + 29.9 × 10⁶ sperm) recovered via electroejaculation. Post-thaw sperm motility and acrosome status did not differ (P > 0.05) between banking methods but fertilization success following IVF of domestic cat oocytes was lower (P < 0.01) for Cath-Vit (35%, 53/150) than straw-frozen (54%, 39/72) samples. For objective 2, veterinarians at three zoos participated in initial field testing of DIY semen banking. Urethral catheterization was attempted with 12 males of five species (tiger, Panthera tigris; lion, Panthera leo; cheetah, Acinonyx jubatus; puma, Puma concolor; bobcat, Lynx rufus). Recovered urethral fluid was vitrified as described above and shipped to the Cincinnati Zoo for post-thaw assessment. Catheterization allowed recovery of urethral fluid (without obvious urine) from nine males; however, only six samples contained any spermatozoa (mean; 0.12 + 0.08 × 10⁶/pellet), and just one vitrified sample (from a cheetah) contained sufficient sperm numbers (0.5 × 10⁶/pellet) and post-thaw motility (10%) for fertilization (13%, 3/24) of domestic cat oocytes. Results suggest that the cath-vit approach may be used as a quick and simple method for semen recovery and cryopreservation in ocelots; however, the occurrence of urine contamination and poor sperm recovery in other felid species...
indicate that further refinement may be required for broader applicability of this cath-vit method for DIY semen banking by zoo veterinarians.

**Key words:** Felids, semen cryopreservation, urethral catheterization, vitrification

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The authors thank the Institute of Museum and Library Services for financial support of this Collection Stewardship grant. The assistance of animal care staff at all participating institutions (Boise Zoo, Caldwell Zoo, Dallas Zoo, Denver Zoo, Houston Zoo, Oklahoma City Zoological Park, San Antonio Zoological Gardens & Aquarium, Texas Zoo, White Oak Conservation) and research staff (Dr. Raquel Gonzalez, Amy Miller) at CREW is greatly appreciated.

**LITERATURE CITED**


CAPSULE ENDOSCOPY AS A NOVEL TOOL FOR GASTROINTESTINAL DISEASE DIAGNOSIS IN A PUMA (Felis concolor)

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Abstract

A 10-yr-old, spayed, female puma (Felis concolor) was presented for intermittent hyporexia and vomition of 6 wk duration. Diagnostics, including baseline imaging and clinical pathology, were performed under anesthesia. No conclusive diagnosis was made. As clinical signs persisted an additional 2 wk, the puma was anesthetized for computed tomography, which identified focal thickening at the pyloroduodenal junction (PDJ) and changes in adjacent soft tissue.

The new option of diagnostic imaging by capsule endoscopy 3 presented an opportunity for this case. This technology is an ingestible camera system contained within a 11 mm × 31 mm capsule that captures 360° high-resolution images and up to 18 hr of data, or approximately 50,000 images. 5 The technology has been used in human medicine for nearly 2 decades, 4 but only recently become available to veterinarians. 1-3 In domestic canines greater than 4.5 kg, it has proven a reliable and safe means of diagnosing lesions throughout the gastrointestinal tract and particularly in the small intestine. 5 The puma had a 24-hr fast from food and then voluntarily ingested the camera in a preferred diet item. The camera was eliminated after approximately 26 hr.

During the camera transit, 17,075 images were obtained in 17.7 hr of study time. As part of the integrated service for the device, images were interpreted by a veterinary internist, which confirmed thickened gastric mucosa and an apparently healing PDJ ulceration. This easy technique produced high quality images and a diagnosis without the need for additional anesthesia or more invasive diagnostic modalities.

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Key words: ALICAM, capsule, endoscopy, Felis concolor, gastrointestinal, puma

LITERATURE CITED


COCCIDIOSIS IN THE ENDANGERED BLACK-FOOTED FERRET (Mustela nigripes): NEW INVESTIGATIONS INTO AN OLD DISEASE

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Abstract

Black-footed ferrets (BFF; Mustela nigripes) were listed as endangered by the International Union for Conservation of Nature in 1967, and became extinct in the wild in the 1980s. Since 1986, multiple institutions have bred BFF in captivity with reintroductions into the wild in their former geographic range. Coccidial enteritis is a major cause of death in young, captive BFF. As a result, fewer captive-bred ferrets may be reared successfully for release to the wild. Consequently, the prevention and control of coccidial outbreaks is an important part of the BFF recovery program. A research program was undertaken to improve in situ and ex situ BFF health through the investigation of the natural history and pathologic features of enteric coccidiosis in this species. Data on morbidity, mortality, clinical signs and shedding of coccidial oocysts from BFF at the Toronto Zoo were collected from 2014-2016. Coccidia isolated from captive BFF fecal samples and identified in a retrospective examination of BFF necropsy tissue from 1999-2016 were characterized using morphometric data and/or molecular diagnostics, and compared to novel and published data from domestic ferrets (Mustela putorius furo). Only one coccidial species, Eimeria cf ictidea, was identified from enteritis and mortality events in captive BFF (1999-2016); mitochondrial whole genome sequencing of this pathogen was achieved. A pilot study performed using the domestic ferret as a model species showed that patent infection can be induced with E. cf ictidea originating from BFF, opening the way for future investigations into the control and treatment of enteric coccidiosis.

Key words: Black-footed ferret, coccidiosis, domestic ferret, Eimeria cf ictidea, Mustela nigripes, Mustela putorius furo

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LITERATURE CITED


SURGICAL LIP-TO-LID TRANSPOSITION CORRECTION AND HUSBANDRY MANAGEMENT FOR SEVERE EYELID COLOBOMA IN A LITTER OF SNOW LEOPARDS (Uncia uncia)

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Abstract

Eyelid coloboma, or agenesis, is a congenital malformation where a section of the eyelid does not form appropriately during embryonic development. There have been multiple reports of eyelid colobomas in snow leopards (Uncia uncia) as well as in other feline species.1-5 Three snow leopard cubs were born to a multiparous dam in May 2016. By 5 days of age, all three cubs were diagnosed with varying degrees of severe eyelid coloboma. Daily separation from the dam, keeper socializations, and topical treatments were initiated on all three cubs at 9 days old and continued until the cubs were 5 mo old. Lip commissure to eyelid transposition surgical corrections were successfully performed on all three cubs at 3-4 mo of age.5 The procedure was performed on one cub at a time and the cub was maintained at the hospital for 10-20 days with daily supervised visits with its siblings. When unsupervised, an Elizabethan-collar was placed on the cub. All cubs were successfully reintroduced with the dam and siblings at the end of the hospitalization. The surgical procedures resulted in functioning eyelids in all three cubs. The consistent human interaction with the cubs before and during the postoperative period made the procedure and its intensive aftercare possible and was crucial to the successful outcome of the surgeries.

Key words: Coloboma, eyelid agenesis, lip-to-lid transposition, snow leopard, Uncia uncia

LITERATURE CITED


RETROSPECTIVE ANALYSIS OF SERUM SYMMETRIC DIMETHYLARGININE (SDMA) CONCENTRATIONS IN CHEETAHS (Acinonyx jubatus) WITH KIDNEY DISEASE

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Abstract

Kidney disease is common among captive cheetahs (Acinonyx jubatus).¹⁵⁻⁶,²⁻⁸,⁹ Serum creatinine is the most common measurement to estimate glomerular filtration rate (GFR), but it is a crude estimate which only increases after significant kidney damage and is affected by extrarenal factors. Symmetric dimethylarginine (SDMA) is a renal biomarker in humans, dogs, and cats, which correlates with GFR and appears to be an earlier, more reliable, and more specific biomarker for kidney disease than creatinine.¹⁻⁴,⁷ In dogs, SDMA increased at 49% GFR reduction (versus 75% for creatinine) and 9.8 mo earlier than creatinine.² In a similar study in cats, SDMA increased 17 mo earlier than creatinine.³ Ninety-two banked serum samples from 11 cheetahs housed at the Oklahoma City Zoo from 1992-2012 were retrospectively analyzed. Histopathology results were available for 10/11 cheetahs and all 10 had histologic renal lesions. General categories of renal lesions included glomerulosclerosis, amyloidosis, inflammatory, and oxalate nephrosis. SDMA immunoassay (IDEXX SDMATM Test) and liquid chromatography-mass spectrometry were measured for validation and compared with creatinine to assess for correlation. Serum creatinine concentrations were determined by enzymatic colorimetric methods and compared with historical measurements. SDMA immunoassay was validated and correlated well with serum creatinine. These data support that SDMA could be a promising renal biomarker in cheetahs. Further research is warranted to investigate whether SDMA might be an earlier indicator of kidney disease in cheetahs than creatinine and whether this assay can be extended to other nondomestic carnivores.

Key words: Cheetah (Acinonyx jubatus), creatinine, kidney disease, SDMA, serum symmetric dimethylarginine

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LITERATURE CITED


PRELIMINARY POPULATION PHARMACOKINETICS OF MELOXICAM IN LION (Panthera leo), CHEETAH (Acinonyx jubatus), AND TIGER (Panthera tigris)

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of analgesics commonly used for the treatment of pain associated with inflammation in both human and veterinary medicine. Meloxicam is a drug product formulated for single-use, subcutaneous injection at a dose of 0.3 mg/kg for the control of postoperative pain and inflammation associated with orthopedic surgery, ovariohysterectomy and castration in domestic felids. The most common indications for use of meloxicam in nondomestic felids include acute (postoperative), and chronic (degenerative osteoarthritis) pain. While NSAIDs are a common treatment option, information regarding their safety, efficacy, and adverse event profiles in nondomestic felids remains limited.3,4 This study represents the first use of population pharmacokinetics (PPK) and sparse data collection to report pharmacokinetic parameters in nondomestic felids. The purpose of this study was to compare and contrast PPK profiles between lions (Panthera leo; n = 22), cheetahs (Acinonyx jubatus; n = 9) and tigers (Panthera tigris; n = 6). Each subject, at their respective zoological institute, received an oral dose of meloxicam (0.1-0.2 mg/kg) and up to three blood samples were voluntarily drawn at time points ranging from 2-4 hr, 8-12 hr, and 20-24 hr. Serum drug concentrations were evaluated at each time point and an appropriate PPK model was established based on the Akaike information criterion (AIC) and -2 log likelihood (-2LL) score. In lions, the maximum serum concentration (Cmax) was 817.9 ng/ml, the corresponding time (Tmax) was 2.1 hr, and a terminal half-life (t1/2) of 5.92 hr. In cheetahs, Cmax was 1412.14 ng/ml, Tmax was 10.03 hr, with a terminal t1/2 of 8.11 hr. Tiger Cmax was 613.7 ng/ml, Tmax was 2 hr, with a terminal t1/2 of 6.31 hr. Oral absorption rate in cheetahs appears to be much slower as compared to both lions and tigers, but all three species were able to achieve therapeutic serum drug concentrations based on the domestic felid pain model.2

Key words: Acinonyx jubatus, meloxicam, NSAID, Panthera leo, Panthera tigris, population pharmacokinetics

ACKNOWLEDGMENTS

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LITERATURE CITED


MEDICALLY IMPORTANT ANTIMICROBIALS IN FOOD-PRODUCING ANIMALS
AFTER 1 JANUARY 2017

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Abstract

The U.S. Food and Drug Administration (FDA) has completed the implementation of Guidance for Industry #213, a process begun in 2013 to transition antimicrobial drugs with importance in human medicine (medically important antimicrobials) that are used in the feed or drinking water of food-producing animals to veterinary oversight and eliminate the use of these products in animals for production (e.g., growth promotion) purposes. The FDA can now report that all affected drug applications have either aligned with the recommendations outlined in GFI #213, or their approvals have been voluntarily withdrawn. As a result of these changes, these products cannot be used for production (e.g., growth promotion) purposes and may only be used under the authorization of a licensed veterinarian. Of the 292 new animal drug applications initially affected by Guidance for Industry #213, 84 were completely withdrawn. Of the remaining 208 applications, 93 applications for oral dosage form products intended for use in water were converted from over-the-counter to prescription status, 115 applications for products intended for use in feed were converted from over-the-counter to veterinary feed directive status. Production (e.g., growth promotion) indications were withdrawn from all (31) applications that included such indications for use.

Key words: Veterinary feed directive

LITERATURE CITED

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FATAL RANAVIRUS OUTBREAK IN A CAPTIVE GROUP OF MELLER’S CHAMELEONS (*Trioceros melleri*)

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Abstract

Five captive-bred Meller’s chameleons (*Trioceros melleri*) presented over a 1-mo period with severe morbidity to acute mortality. Clinical signs included lethargy, dehydration, poor appetite, dysedcdysis, mucoid ocular discharge, and skin vesicles. Empirical treatment, including famciclovir (20 mg/kg p.o., s.i.d.), ceftazidime (30 mg/kg s.c., every 3 days), and meloxicam (0.2 mg/kg once s.c.), gavage feeding, and subcutaneous fluid administration, was pursued without obvious clinical effect. All cases displayed rapid clinical progression, ending in natural death or euthanasia a maximum of 11 days after the onset of clinical signs. Gross necropsy lesions included mild coelomic effusion and petechiation of the tongue and kidneys. Histopathologic changes included necrosis of the spleen, liver, kidney, adrenal tissue, and nasal cavity and basophilic intracytoplasmic inclusion bodies in the liver and nasal mucosa. Viral quantitative polymerase chain reaction (PCR) from each individual was positive for ranavirus but negative for herpesvirus and adenovirus. Further, ranavirus sequencing was consistent with the known frog virus 3 strain present in the Eastern box turtle (*Terrapene carolina carolina*) group housed at the same facility. The two species had no identifiable direct or indirect sources of contact to facilitate transmission. Further, the Meller’s chameleons were housed in a mesh sided enclosure immediately adjacent to a similar enclosure housing an Oustalet’s chameleon (*Furcifer oustaleti*). That animal remained asymptomatic and PCR negative until its death of unrelated causes 8 mo after this outbreak. To the authors’ knowledge, this case series is the first to document ranavirus associated disease in the Chamaeleonidae family.

Key words: Meller’s chameleon, ranavirus, *Trioceros melleri*

ACKNOWLEDGMENTS

The authors would like to thank the animal care staff at the Maryland Zoo in Baltimore for the diligent care they provided for these chameleons.
LITERATURE CITED

CANINE DISTEMPER VIRUS OUTBREAK IN CAPTIVE LINNAEUS’S TWO-TOED SLOTHS (Choloepus didactylus): CLINICAL AND PATHOLOGIC FINDINGS AND RESPONSE TO VACCINATION

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Abstract

An outbreak of canine distemper virus (CDV) caused the death of five adult Linnaeus’s two-toed sloths (Choloepus didactylus) living in separate enclosures in a building at a private zoo in eastern Tennessee during a 2-wk period. Clinical signs included oral and nasal discharge and ulcerations, diarrhea, lethargy, and anorexia. Diagnosis was confirmed via necropsy, histopathology, immunohistochemistry, virus isolation, and PCR. Viral sequencing identified the strain to be consistent with a new CDV lineage currently affecting domestic dogs and wildlife in Tennessee.3,4 Canine distemper has not been previously reported in any species in the super order Xenarthra.1,2 Seven sloths (three surviving the outbreak, and four animals added after the outbreak) were sedated, examined, and vaccinated with a recombinant CDV vaccine (Recombitek C3, Merial, 1 ml s.c.) on day 0, and again on day 21. Blood was collected and CDV antibody titers were measured prior to initial vaccination in all seven sloths and on day 49 in four sloths (two sloths died of non-CDV disease before day 49 and an adequate sample could not be obtained in one sloth). Serology revealed negative titers (≤ 1:32) on day 0 in six of seven sloths, and titers increased by day 49 in three of four sloths with seroconversion in two sloths and a 32-fold increase in one sloth. No adverse effects of vaccination were observed. Based on this outbreak and serologic findings post vaccination without adverse effects, the authors recommend recombinant CDV vaccination in sloths exposed to known carriers of CDV.

Key words: Canine distemper virus, Choloepus didactylus, Recombitek, serology, sloth, vaccination

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LITERATURE CITED


CLINICAL COWPOX INFECTION IN TWO GIANT ANTEATERS (*Myrmecophaga tridactyla*)

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Abstract

Two giant anteaters (*Myrmecophaga tridactyla*), a 5-yr-old female and a 7-yr-old male, were diagnosed with clinical Orthopoxvirus (cowpox) infection in 2014 and 2016, respectively. Clinical signs were similar, including inappetence and the development of multifocal ulcerative and vesicular lesions extending along their tongues, rostrums and throughout their oral cavities. Disseminated skin lesions were also found over their bodies, mostly affecting their feet, flanks and genital areas. Polymerase chain reaction (PCR) assays performed on dry swabs of these lesions confirmed the clinical suspicion of Orthopoxvirus (suspected cowpox) infection. Cowpox virus is endemic in Europe, where wild rodents are the main reservoir hosts, but clinical disease may occur in opportunistic hosts with felids and elephants amongst the species most often affected.1 In a previous report from 1973, the virus affected two giant anteaters, both of which died.2 In this case, the first animal made a full recovery after it was anaesthetised on seven occasions in order to administer nonspecific supportive care, including parenteral fluid administration, antibiosis, pain relief, anti-inflammatory medication and supplementary feed via an orogastric tube. Acyclovir was administered at a dose extrapolated from dogs and cats. The second animal died under anesthesia during initial assessment and postmortem revealed cowpox lesions that were partially obstructing its glottis. The two outcomes in these cases made it possible to examine the full extent of the lesions present on a postmortem specimen, as well as to show that, in some cases, intensive treatment may result in full recovery from this serious zoonotic disease.

Key words: Cowpox, giant anteater, *Myrmecophaga tridactyla*, Orthopoxvirus

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LITERATURE CITED


GASTROINTESTINAL DISEASE ASSOCIATED WITH NON-albicans Candida SPECIES IN BIRDS

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Abstract

Candida albicans is the most commonly described yeast of clinical interest; however, non-albicans Candida infections are an emerging issue in human health care. These species are markedly more resistant to antifungals.2,4 Six cases of non-albicans Candida species were identified in six birds with gastrointestinal signs. The gastrointestinal signs included diarrhea, regurgitation, and weight loss, and were often concurrent or identified secondary to other systemic signs including dermatitis, weakness, and torticollis. Candida glabrata was identified in an Amazon parrot (Amazona sp.), a ringneck dove (Streptopelia risoria), a blue and gold macaw (Ara ararauna), and two cockatiels (Nymphicus hollandicus). Candida krusei was identified in a white-crowned pionus parrot (Pionus senilis). Three cases resolved after treatment, and two birds died. Fungal culture and MALDI-TOF mass spectrometry identification was correlated with fecal and/or crop cytology, and DNA sequencing was used in one case. Most cases had a history of prior antibiotic exposure. Recent human data describes a shift in species distribution away from C. albicans dominance and toward other species, including C. glabrata and C. krusei.2 Both species are a normal component of human and bird mycobiota, which may emerge to cause disease, especially with disruption of normal gut ecology due to prior antibiotic use.1,3

Key words: Bird, Candida albicans, Candida glabrata, Candida krusei, emerging disease, yeast

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LITERATURE CITED

TOOLS FOR THE EXOTIC ANIMAL INDUSTRY FROM THE SECURE ZOO STRATEGY PROGRAM

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Abstract

The Secure Zoo Strategy (SZS) was introduced to the American Association of Zoo Veterinarians (AAZV) community at the 2015 annual conference. SZS is an on-going effort of the Zoo and Aquarium All Hazards Preparedness (ZAHP) Response and Recovery Fusion Center, a cooperative agreement between the U.S. Department of Agriculture (USDA) and the Association of Zoos and Aquariums (AZA)

Based upon the successful industry-driven Secure Milk, Secure Pork and Secure Poultry programs, SZS provides a path forward for planning with the ultimate goal for business continuity in the face of foot-and-mouth disease (FMD) outbreak. The SZS combines the expertise of many members of AAZV, AZA, USDA, Zoological Association of America (ZAA), state veterinarians, and other stakeholders to produce guidance addressing reasonable alternatives to traditional mitigation strategies for the industry in the event of a foreign animal disease (FAD) or other hazard.

SZS is a process to develop preparedness and response plans for FAD events. It encourages the establishment of specific goals, which drive the planning process. Goals will differ between exotic animal industry facilities, and each facility should share their goals up front with the stakeholders involved in their planning process.

This presentation will share the basic steps described in the SZS, and demonstrate a mapping tool that uses Google Earth as a common platform. The legend has been developed to utilize the same terminology that State Animal Health Officials are already familiar with from the other secure programs. This virtual view of the facility will be important for planning efforts, and critical if FMD is detected near the facility.

State and Federal governments, and the agricultural industry have dedicated an incredible amount of time and resources to FMD disease planning. Adoption of a SZS, with its recommended steps, will increase the level of preparedness in a facility and open or strengthen lines of communication among responders, with the overall goal of business continuity and recovery.

Key words: Foreign animal disease, hazards, preparedness
SUPPORTING ALTERNATIVES TO TRADITIONAL ELEPHANT TRAINING METHODS IN SOUTHEAST ASIA

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Abstract

In Asia about 16,000 elephants are currently maintained in captivity for a variety of purposes: logging, tourism, cultural and religious activities, and transportation. The training that they have received for these tasks has always been a subject of discussion because of the aggressive methods often used. The people that handle the elephants, also known as mahouts, have ancient training methods. These methods have evolved into dangerous labor that is sometimes characterized by firm and rough interactions with the elephants. It is surprising that this practice of intimidation has not changed over the years despite the long list of injuries and fatalities of mahouts, bystanders, and elephants alike, arguably attributed directly to the use of these practices. For this reason, Africam Safari and The Golden Triangle Asian Elephant Foundation have been teaching mahouts from seven countries of Southeast Asia how to handle their elephants through the use of safe and harmless methods. The goal is to convince the elephants to cooperate voluntarily without being threatened or harmed in order to receive the proper care they need. The most difficult task of this positive reinforcement project has been to convince the mahouts, whom are talented and with invaluable skills, to relinquish the practices they have been taught and have followed for many centuries. Nonetheless, many of the mahouts have been very receptive and remarkable results have been achieved with both the mahouts and their elephants. The goal is to upgrade the perception of the proper care and handling of the elephants. The primary aim is to encourage the use of effective alternatives that can be incorporated into the culture and traditions of the mahouts and then be transmitted to future generations.

Key words: Elephant, Elephas maximus, mahout, positive reinforcement

ACKNOWLEDGMENTS

The author would like to thank the other members of the positive reinforcement project team: Dr. Khyne U Mar, John Roberts, and Rodrigo Salas, DVM.
USE OF INTERLEUKIN RECEPTOR ANTAGONIST PROTEIN (IRAP) IN A MULTI-MODAL THERAPEUTIC REGIME FOR OSTEOARTHRITIS IN AN ASIAN ELEPHANT (*Elephas maximus*)

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Abstract

A ~41-yr-old female Asian elephant (*Elephas maximus*) experiencing forelimb stiffness and decreased range of motion was diagnosed with bilateral carpal osteoarthritis (OA). Symptomatica and OA specificb treatments had mixed success in alleviating clinical signs.

Standing sedation combined with local anesthesia was employed to deliver ultra-sound guided carpal articular injections using an autologous conditioned serum product, interleukin receptor antagonist protein (IRAP)c combined with hyaluronic acid and amikacin. IRAP was delivered into radiocarpal, intercarpal, and carpometacarpal joints bilaterally at 2 wk intervals for three treatments in each joint.1 Symptomatic treatment was assessed using keeper photos, videos, stiffness and mobility charts, and daily observations. Disease treatment was assessed using serial monitoring of complete blood counts, serum chemistries, protein electrophoreses (EPH), amyloid A (SAA), and haptoglobin4 levels; carpal radiographs; and articular prostaglandin E2 (PGE2)e values.

Following articular injections, increased discomfort lasted ~24-48 hr, then resolved. Within 2 mo of completing therapy, improved range and speed of motion were evident. Decreased inflammation was evidenced by heat degraded protein, SAA, haptoglobin, and PGE2 levels. Improved clinical signs and bloodwork parameters lasted ~5-6 mo, at which point IRAP was repeated.

Based on the positive symptomatic and disease response noted, autologous therapy using IRAP is recommended for consideration for carpal osteoarthritis in elephants. Serum and joint inflammatory markers were instrumental in gauging response to treatment, progression of disease, and determining when additional treatments were indicated, and should be included in the diagnostic and therapeutic approach to osteoarthritis.

aCosequin, Veterinary Sciences, Inc, Lancaster, South Carolina 29720 USA; Gabapentin, Actavis Pharma, Inc, Parsippany, New Jersey 07054 USA; Ibuprofen, Amneal Pharmaceuticals, Bridgewater, New Jersey 08807 USA; Gastrogaurd, Merial, Inc, Duluth, Georgia 30096 USA 
bLegend, Merial, Inc, Duluth, Georgia 30096 USA; Adequan, Luitpold Pharmaceuticals, Inc, Animal Health Division, Shirley, New York 11967 USA
Interleukin receptor antagonist protein, Dechra Veterinary Products, Overland Park, Kansas 66211 USA
Protein electrophoresis, serum amyloid A, and haptoglobin measurements performed at Acute Phase Protein Laboratory, Division of Comparative Pathology, Miller School of Medicine, University of Miami, Miami, Florida 33136 USA
Prostaglandin E2 measurements performed at the Orthopedic Research Center, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523 USA

**Key words:** Arthritis, Asian elephant, *Elephas maximus*, inflammatory markers, interleukin receptor antagonist protein, treatment

**ACKNOWLEDGMENTS**

The authors would like to thank the elephant house staff and veterinary staff at National Zoological Park, Dr. Betsy Herrelko, Nikki Phillips, and Karen Dailey for their assistance in the care and treatment of this elephant.

**LITERATURE CITED**

TUBERCULOSIS CAUSED BY *Mycobacterium orygis* IN A GREATER ONE-HORNED RHINOCEROS (*Rhinoceros unicornis*): FIRST REPORT IN THE WESTERN HEMISPHERE

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Abstract

*Mycobacterium orygis*, a member of the *Mycobacterium tuberculosis* complex (MTBC), has been isolated predominantly from hoofstock in eastern Africa and the Arabian Peninsula, and occasionally in cattle, rhesus monkeys, humans, and greater one-horned (GOH) rhinoceros (*Rhinoceros unicornis*) in southern Asia, with documented zoonotic and zooanthroponotic transmission.1,5,7 Typically, rhinoceros develop chronic progressive respiratory disease as a result of MTBC infection.3 This report describes the postmortem diagnosis of tuberculosis caused by *M. orygis* in a GOH rhinoceros imported from India who developed hindlimb paresis due to neural granulomatosis. The postmortem use of dual path platform technology (DPP® VetTB Assay)a and multi-antigen print immunoassays (MAPIA)a to detect specific anti-MTBC antibodies prior to culture results on serum collected just before death from this rhinoceros allowed for appropriate preventive and management protocols to be initiated in the herd, highlighting the potential of serodiagnostics for antemortem diagnosis of tuberculosis in rhinoceros (Figures 1 and 2).2,8 *Mycobacterium* genus-specific PCR assaysb followed by direct sequencing allowed timely confirmation of the serology results.4,6 Banked serum collected up to 6 yr prior to death correlated closely with the course of clinical disease, suggesting latent infection originating from India.9 This is the first isolation of *M. orygis* within the western hemisphere, further exemplifying the need for more stringent testing prior to international shipment, and the urgency for validated antemortem MTBC screening assays in rhinoceros species.

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b Washington Animal Disease Diagnostic Lab (WADDL), College of Veterinary Medicine, Washington State University, Bustad Hall Room 155N, Pullman Washington 99164 USA

**Key words:** Greater one-horned rhinoceros, *Mycobacterium orygis*, neurologic, *Rhinoceros unicornis*, serodiagnostics, tuberculosis
ACKNOWLEDGMENTS

The authors would like to thank veterinary and animal management staff at The Wilds for their incredible care of this rhinoceros prior to its death. The authors also acknowledge the staff and laboratory technicians at Washington Animal Disease Diagnostics Laboratory and National Veterinary Services Laboratory for their diligent work with the sample testing for this case.

LITERATURE CITED


**Figure 1.** Antibody response against eight recombinant *Mycobacterium tuberculosis* complex (MTBC) protein antigens (ESAT6, CFP10, MPB64, MPB70, MPB83, E6/P10, DID38, and DID65) and two native antigens (*M. bovis* purified protein derivative, B-PPD, and *M. bovis* culture filtrate, MBCF) detected by multi-antigen print immunoassay (MAPIA) in serum samples collected between 2010 and 2016 from a greater one-horned rhinoceros bull diagnosed in 2016 with tuberculosis from *M. orygis* infection.

**Figure 2.** Evolution of antibody response against the major *Mycobacterium tuberculosis* complex protein antigens, MPB83 and CFP10/ESAT6, detected by DPP VetTB assay in serum samples collected between 2010 and 2016 from a greater one-horned rhinoceros diagnosed in 2016 with tuberculosis from *M. orygis* infection. Optical reader value (Y-axis) reflectance measured in relative light units (RLU); broken line indicates cut-off value of 40 RLU, equal to threshold of visual test interpretation.
SUDDEN DEATH IN THREE SOUTHERN WHITE RHINOCEROS (Ceratotherium simum simum) SECONDARY TO PRESUMPTIVE Clostridium perfringens ENTEROTOXEMIA

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Abstract

Within 3 mo, three female Southern white rhinoceros (Ceratotherium simum simum) from a private conservation center died suddenly. Ages ranged from 4-10 yr and two had young calves. All individuals were acting normal shortly before death. Biochemical panels, complete blood counts, urine, milk, and fecal analyses were unremarkable. Individuals had mild abdominal transudate, splenic congestion, adrenal hemorrhage, and multifocal intestinal congestion. Analysis of stomach contents for toxins, virus isolation, negative stain electron microscopy, and blood cultures were unrewarding. Gammaherpesviruses were identified via polymerase chain reaction (PCR) from the jejunum and spleen of two rhinoceros. Histologic findings were mild mucosal congestion and hemorrhage in the small and large intestinal mucosa, consistent with acute enteric and systemic shock. Rapid intestinal sample acquisition was challenging and tissue autolysis precluded complete histologic examination of the gut. Clostridial enterotoxemia was suspected, based on the cumulative postmortem findings. Heavy growth of Clostridium perfringens genotype A was cultured from all rhinoceros; however, available toxin tests yielded negative results. Based on the presumptive diagnosis, the entire rhinoceros herd was vaccinated with Clostridium perfringens toxoids. Serum was obtained from all rhinoceros at the time of initial vaccination, time of vaccine booster, and 1 mo following to evaluate antibody response. All rhinoceros, with the exception of a young calf, showed positive serologic response to vaccination. Many of the rhinoceros developed mild vaccine site reactions, consisting of local soft tissue swelling and occasional abscessation. Vaccination was otherwise well tolerated. Since completion of the vaccine series, no additional cases have occurred.

Clostridium perfringens types C&D toxoid, Professional Biological Company, Denver, Colorado, 80216 USA, 2 ml s.c., 2 series, 4-wk interval.
Clostridium perfringens type A toxoid, Novartis Animal Health US, Inc, Larchwood, Iowa, 51241 USA, 2 ml s.c., 2 series, 4-wk interval.
BIO K 291-Monoscreen AbELISA Clostridium perfringens alpha toxin/blocking, Bio-X Diagnostics S.A., Rochefort, 5580 Belgium

Key words: Ceratotherium simum simum, Clostridium perfringens, enterotoxemia, white rhinoceros

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colleagues, Dr. Michael Kinsel, Dr. James Wellehan, Dr. Michele Miller, and Dr. Charles van Niekerk for their diagnostic assistance and input on these cases. Additionally, the authors would like to thank Dr. Dubraska Diaz-Campos, Dr. Francisco Uzal, and Dr. Glenn Songer for offering expert advice related to clostridial diseases and performing *Clostridium* cultures, genotyping, and toxin assays.
NEGATIVE EFFECTS OF ANALGESIC AND ANESTHETIC DRUGS ON SPERM MOTILITY: IMPLICATIONS FOR ASSISTED BREEDING IN MANAGED RHINOCEROS

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Abstract

Drugs utilized for immobilization for assisted breeding may have negative effects on sperm motility. Opioid receptors have been detected on the head, neck, and tail of sperm cells of humans, horses, and boar. The mu and delta opioid receptors modulate sperm motility; however, interspecies variation exists. Butorphanol stopped sperm motility in human in vitro studies, while other opioids, such as fentanyl, had partial inhibitory activity. Domestic horses and cattle were used to develop an in vitro model evaluating drugs utilized for semen collection in rhinoceros. Semen was collected from stallions with an artificial vagina and from bulls using electro-ejaculation. Semen concentrations were standardized to a minimum 60 million sperm cells/ml. Butorphanol (µ and κ opioid partial agonist/antagonist), naloxone (opioid antagonist), xylazine (alpha-2 agonist) and detomidine (alpha-2 agonist) were added to aliquots of sperm at concentrations from 0.01 µg/ml to 400 µg/ml and incubated for 240 min. Computer-assisted sperm analysis (CASA) was used to determine sperm motility as the percent of progressive motility at drug exposure and specific time intervals thereafter. Butorphanol, detomidine, and xylazine had significant negative effects on sperm motility with increasing drug concentrations. Naloxone had no effects on progressive sperm motility at all drug concentrations evaluated. Opioid drugs (butorphanol and etorphine) were detected in stored seminal plasma from immobilized black (Diceros bicornis), southern white (Ceratotherium simum simum), and greater one-horned (Rhinoceros unicornis) rhinoceros, indicating the potential for negative effects of these drugs on sperm motility. Comparison of drug concentrations from additional serum and seminal plasma samples are pending.

Key words: Artificial insemination, opioids, rhinoceros, sperm motility

ACKNOWLEDGMENTS

The authors would like to thank the staff of Auburn University North Beef Unit and Horse Reproduction Unit for assistance in semen collection for the in vitro analysis portion of this study.

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CHARACTERIZATION AND ADMINISTRATION OF ALLOGENEIC BLOOD-DERIVED MESENCHYMAL STEM CELLS IN AN AFRICAN ELEPHANT (Loxodonta africana) WITH SEvere OSTEOARTHRITIS

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Abstract

Arthritis is a common condition in captive elephants for which few curative remedies are available or effective. The advent of cellular therapies as a novel treatment for osteoarthritis in multiple species prompted the investigation of the utility of this therapy in this population. Mesenchymal stem cells (MSC) are derived from stromal cells located in adult bone marrow or adipose tissue; however, acquisition of these tissues in an elephant can be problematic. This report describes the successful derivation and expansion of MSC from the blood of a 38-yr-old female African elephant (Loxodonta africana) and subsequent intravenous delivery of these cells for the purpose of ameliorating severe clinical signs of osteoarthritis of the stifle joint. The cells were confirmed to be MSC via surface marker phenotype, trilineage differentiation, and function assessed via lymphocyte suppression. Clinical signs in this elephant had been present for approximately 15 yr and had progressed despite conventional therapy involving medications and physical therapy. The elephant had deteriorated to circumducting its leg most of the time with only 11% bending based on hourly observations. Following stem cell therapy the elephant displayed significant reduction in clinical signs of osteoarthritis as determined by thermal imaging, ability and willingness to perform physical therapy and increased range of motion in the joint with up to 91% bending of the stifle by 2 mo after treatment. Improvement was sustained throughout the following year and deterioration again noted by decreased bending of the stifle 12-13 mo after initial treatment. At this time allogeneic MSC isolated from umbilical cord blood of an African elephant were expanded and administered intravenously. The second injection was well tolerated and followup monitoring will be performed and assessed as for the previous injection. MSC therapy is feasible in this species and could represent a novel therapy for the treatment of a common and problematic disorder in the aging population of elephants under managed care.

Key words: Elephant, Loxodonta africana, osteoarthritis, regenerative medicine, stem cell

ACKNOWLEDGMENTS

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LITERATURE CITED

IN-DEPTH ANALYSIS OF THE VITAMIN D AND CALCIUM STATUS OF ASIAN ELEPHANTS (Elephas maximus) MANAGED IN A NORTHERN TEMPERATE CLIMATE

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Abstract

Little is known about vitamin D and calcium metabolism in elephants, and reports of disease in resulting from imbalances in calcium homeostasis exist. This study monitored analytes associated with vitamin D and calcium status in a herd of healthy adult Asian elephants (Elephas maximus) maintained in Syracuse, NY for 1 yr and correlated that information with dietary vitamin D and calcium intake and ultraviolet light levels. It is hypothesized that seasonal differences in vitamin D status would be observed, regardless of diet, with maximum values during the summer and deficient values during the winter. Serum samples were analyzed monthly for 25(OH)D$_2$/D$_3$, 1,25(OH)$_2$D$_2$/D$_3$, 24,25(OH)$_2$D$_2$/D$_3$, parathyroid hormone, ionized calcium, and minerals (calcium, phosphorus, magnesium). Diet samples (hay and pelleted formula) were analyzed for vitamin D$_2$ and D$_3$, and minerals (calcium, phosphorus). Daily ultraviolet light levels were obtained from the Colorado State University’s UVB Monitoring and Research Program station in Geneva, NY (latitude: Geneva 42.868°N, Syracuse 43.048°N).

The major contribution to the total serum 25(OH)D was vitamin D$_2$, indicating that forage and not cutaneous synthesis was the main source of vitamin D in this herd. Seasonal effects on vitamin D status were not observed in any of the elephants despite significant seasonal variations in ultraviolet irradiance. Total 25(OH)D levels in all elephants were markedly lower than those reported in other studies (mean 7.02 ± 0.55 ng/ml). This study provides important new information regarding vitamin D metabolism in the Asian elephant, and will serve as a basis for future investigations to determine normal values, monitoring recommendations, and nutritional requirements.

Key words: Asian elephant, calcium, diet, Elephas maximus, ultraviolet light, vitamin D

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LITERATURE CITED


VISCERAL LEPIDOPTERISM IN CAPTIVE OTARIIDS DUE TO MIGRATION OF CATERPILLAR SETAE ASSOCIATED WITH SYSTEMIC VASCULOPATHY AND DEATH

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Abstract

Lepidopterism refers to pathology caused by invertebrates of the order Lepidoptera, moths and butterflies. Pathology is produced by irritant chitinous hairs or setae found on the larval exoskeleton. Caterpillar setae have been associated with irritant dermatitis, ophthalmitis, and oropharyngitis in humans and dogs following contact and/or migration of these barbed chitinous structures. In recent years, migrating caterpillar setae were linked to reproductive losses and mortality in horses in the United States and Australia, the first demonstration of visceral lepidopterism in any species. Visceral lepidopterism has not been reported in any other species. In December 2015, an 8-yr-old captive-born California sea lion (Zalophus californianus) at Taronga Zoo died following 3 wk of nonspecific illness. Postmortem examination revealed a marked vasculocentric mural granulomatous gastro-colitis associated with foreign bodies consistent in morphology with migrating chitinous invertebrate setae. Some setae were observed penetrating submucosal blood vessels. Death was attributed to major vascular and circulatory events: pancreatic apoplexy, centrilobular hepatic necrosis, and renal thromboembolic fibrinoid vasculitis. These findings prompted a review of prior deaths in otariids at Taronga Zoo. Between 1998 and 2017, 10/19 nonneonatal deaths had microscopic evidence of visceral caterpillar setae migration and associated pathology including 2/2 California sea lions, 5/6 Australian sea lions (Neophoca cinerea), and 3/8 long-nosed fur-seals (Arctocephalus forsteri). Microscopically, the setae are morphologically consistent with those shed by bag-shelter moth (Ochrogaster lunifer) larvae which are known to cause visceral lepidopterism in Australian horses. O. lunifer caterpillars have subsequently been found in and around otariid enclosures at Taronga Zoo.

Key words: Bag-shelter moth, lepidopterism, Ochrogaster lunifer, Otariid, Otariidae, vasculopathy

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LITERATURE CITED


ARE SALAMANDERS SAFE? HEALTH ASSESSMENT OF THE SILVERY SALAMANDER (*Ambystoma platineum*) IN VERMILION COUNTY, ILLINOIS USA PRIOR TO AND DURING A RANAVIRUS MORTALITY EVENT

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Abstract

Amphibian ranavirus and chytridiomycosis infections are reportable to the OIE due to their impacts on population stability. Both pathogens have been detected in clinically affected individuals in Illinois. The state-endangered silvery salamander (*Ambystoma platineum*) is considered a species of conservation concern; however, information on baseline silvery salamander health and pathogen susceptibility has not been reported. The purpose of this study was to characterize the health and disease status of silvery salamanders through physical examination and PCR screening for ranavirus and chytridiomycosis. Adult silvery salamanders (*n* = 84) were captured at 10 ponds in Vermilion County, Illinois during spring emergence in March, 2016. A frog virus 3-like ranavirus was detected in seven individuals (8.3% prevalence). Ranavirus occurrence was significantly associated with the presence of raised skin nodules (*P* = 0.02). From May 1 to June 22, approximately 300 silvery salamander larvae, representing over 80% of the larval population, were found dead. Gross necropsy findings included hemorrhages and subcutaneous edema. A frog virus 3-like ranavirus was PCR amplified from liver and kidney samples (*n* = 50, 100% prevalence). Surviving metamorphs (*n* = 14) were captured in June. Five individuals tested PCR positive for ranavirus (35% prevalence). Two displayed hemorrhages, one had traumatic injuries, and two were aclinical. This study demonstrates that disease may pose a threat to silvery salamander conservation in Illinois, and underscores the need for continued health assessment in this species. This health assessment framework can be applied to other imperiled salamander populations to assess threats to conservation, and monitor for emerging infectious diseases such as *Batrachochytrium salamandrivorans*.

**Key words:** *Ambystoma platineum*, health assessment, ranavirus, silvery salamander

ACKNOWLEDGMENTS

The authors thank the herpetologists at the Illinois Natural History Survey for their assistance with salamander trapping and sample collection.

LITERATURE CITED


SARCOCYSTOSIS IN A FLOCK OF THICK-BILLED PARROTS (Rhynchopsitta pachyrhyncha), 2005-2016: MORBIDITY, MORTALITY, DIAGNOSTICS, AND MANAGEMENT STRATEGIES

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Abstract

The Wildlife Conservation Society maintains one of the largest captive flocks of thick-billed parrots (Rhynchopsitta pachyrhyncha). Since initial diagnosis in 2005, sarcocystosis has led to 51.7% of mortalities in birds over 30 days of age. In all cases tested by PCR, Sarcocystis falcatus is consistently identified, and disease predominantly impacts younger animals (median age 5.62 yr; age range 0.19-26.09 yr). Administration of antiparasitic medications prior to development of respiratory signs prolonged life in infected birds, but disease was uniformly fatal until utilization of a three-drug combination (pyrimethamine, trimethoprim-sulfamethoxazole, and ponazuril). This protocol requires in excess of 6 mo of therapy to achieve clinical resolution, resulting in ongoing efforts to develop alternate treatments. Plasma creatine kinase activity appears to be the most useful minimally invasive test to diagnose infection, as well as a means to monitor response to therapy. PCR for apicomplexan organisms on antemortem whole blood, blood smears, or blood cards helps confirm suspected cases, but may be misleading when assessing response to therapy or resolution of clinical disease. Historically, preventive measures focused on excluding Virginia opossums (Didelphis virginiana), the definitive host for Sarcocystis falcatus, from the parrot areas and removal of opossums from zoo grounds. Despite these management efforts, birds continued to develop sarcocystosis resulting in additional steps, including modifying food stations to exclude potential arthropod paratenic hosts. Additionally, prophylaxis trials with diclazuril were implemented. Given treatment and antemortem diagnostic challenges, preventing exposure to Sarcocystis spp. and their hosts is essential for continuing ex-situ conservation of thick-billed parrots.

Key words: Rhynchopsitta pachyrhyncha, Sarcocystis falcatus, thick-billed parrot

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The authors thank the animal care staff, veterinary, pathology, and histology technicians at the Queens Zoo and the Bronx Zoo Wildlife Health Center for their care and treatment of these animals.
EVALUATION OF A NOVEL HERPESVIRUS AS A SENTINEL FOR POPULATION HEALTH IN ENDANGERED BLANDING’S TURTLES (Emydoidea blandingii)

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Abstract

While herpesvirus outbreaks have been associated with high morbidity and mortality in populations of captive Emydid chelonians worldwide, novel herpesviruses have also recently been identified in apparently healthy free-ranging Emydid populations.2-4,6,7,10 The clinical significance of this finding in the absence of an outbreak is currently unknown. The authors hypothesize that herpesvirus prevalence may be used as a sentinel of population health due to the virus’ propensity to recrudesce in times of stress or concurrent disease.1,5 Blanding’s turtles (Emydoidea blandingii), an endangered species in Illinois, have experienced range-wide declines because of habitat loss, degradation, and fragmentation.8,9 A novel herpesvirus, Emydoidea herpesvirus 1 (EBHV-1), was identified in Blanding’s turtles in DuPage County, Illinois in 2015. Subsequently, the investigators developed a highly sensitive and specific quantitative TaqMan PCR assay to target the DNA polymerase gene. Combined oral-cloacal swabs were collected from radiotelemetered and trapped Blanding’s turtles in DuPage (n = 60 turtles) and Lake (n = 81 turtles) County from May-September 2016. Radiotelemetered females had a significantly higher prevalence of EBHV-1 in May (24.4%) than June (3.7%), July (0%), August (0%), or September (7.7%) (P = 0.001). This corresponds to the onset of nesting and may be associated with increased physiologic demands; however, all positive turtles were negative in subsequent months. Furthermore, there were no clinical signs associated with any turtle at the time they were detected with EBHV-1. This investigation is the critical first step to characterizing the implications of EBHV-1 on Blanding’s turtle population health and identifying management changes that may improve sustainability.

Key words: Blanding’s turtle, chelonian, Emydoidea blandingii, herpesvirus, polymerase chain reaction

LITERATURE CITED


DRIVERS AND IMPACTS OF SINGLE AND CO-PATHOGEN OCCURRENCE IN FREE-LIVING EASTERN BOX TURTLES (*Terrapene carolina carolina*) IN ILLINOIS AND TENNESSEE USA, 2013-2016

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Abstract

Infectious diseases are increasingly detected in free-living herptiles, but their clinical significance is not always obvious. The impacts of Terrapene herpesvirus 1 (TerHV1) and adenovirus (AV) on Eastern box turtle (*Terrapene carolina carolina*) health are largely unknown. Other pathogens, such as frog virus 3 (FV3), have known effects (mortality), but epidemiology in free-living populations is poorly characterized. This study describes comprehensive health assessments, including qPCR testing for three pathogens simultaneously, in 724 free-living Eastern box turtles sampled from 2013-2016 in Illinois and Tennessee, USA. The purpose was to identify factors associated with qPCR detection of TerHV1, AV, FV3, and co-infections. Single pathogens were detected in 249 turtles (15 FV3, 196 TerHV1, 35 AV). Co-infection was detected in 18 turtles (3 FV3 & TerHV1, 15 TerHV1 & AV). Season, state, substrate, location, sex, age class, nasal discharge, oral plaques, and increases in leukocyte count, heterophil count, and monocyte count were significantly associated with qPCR detection of FV3 ($P < 0.05$). Year, season, physical examination abnormalities, increases in PCV and TS, and decreases in lymphocyte count and monocyte count were associated with TerHV1 detection ($P < 0.05$). Sex, year, age class, weight, and body condition were associated with AV detection ($P < 0.05$). Sex and increased lymphocyte count were associated with co-detection of TerHV1 and AV ($P < 0.05$). This is the first large-scale study to document host and environmental factors influencing single and co-pathogen dynamics in Eastern box turtles. It provides a valuable starting point for understanding the drivers and impacts of disease in free-living box turtles.

Key words: Adenovirus, Eastern box turtle, frog virus 3, ranavirus, *Terrapene carolina carolina*, Terrapene herpesvirus 1

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LITERATURE CITED


RETROSPECTIVE CHARACTERIZATION OF REPRODUCTIVE TRACT LESIONS IN RELATION TO AGE, PARITY, AND CONTRACEPTION IN CAPTIVE FEMALE SUIDAE AND TAYASSUIDAE

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Abstract

Reproductive management in zoos often requires contraception or physical separation of sexes to ensure long term viability in captive populations.4 Correlation between reproductive life histories and uterine pathology has been documented in zoo ungulates, including elephants and rhinoceroses.1-4 Information on the effects of parity, age, and contraceptive use on lifetime reproductive health in captive pig (Suidae) and peccary (Tayassuidae) species is sparse. This study systematically evaluated the reproductive tract tissues and comprehensive reproductive histories from female babirusa (Babyrousa babyrussa, n = 6), red river hog (Potamochoerus porcus, n = 10), Chacoan peccary (Catagonus wagneri, n = 5), Visayan warty pig (Sus cebifrons, n = 5), and common warthog (Phacochoerus africanus, n = 26). Age, parity, time-since-last-parturition (parturition gap), contraception exposure, lesion prevalence, and endometrial lesion grade were recorded. Reported contraceptives included PZP, progestins and deslorelin. Red river hogs were most commonly contracepted (42.9%). Babirusa had the highest prevalence of follicular cysts (66.7%), endometrial atrophy (33.3%), endometrial hyperplasia (66.7%), pyometra (66.7%), and uterine neoplasia (66.7%, leiomyoma only). Red river hogs had the highest prevalence of adenomyosis (30.0%). Warthogs had the highest prevalence of follicular cysts (66.7%), endometrial atrophy (33.3%), endometrial hyperplasia (66.7%), pyometra (66.7%), and uterine neoplasia (66.7%, leiomyoma only). Red river hogs had the highest prevalence of adenomyosis (30.0%). Warthogs had the highest metritis (41.1%) and the second highest uterine neoplasia (23.5%, leiomyoma and vaginal leiomyoma) prevalence. Overall, age was positively correlated with pyometra occurrence (P = 0.0167). Contraceptive use positively correlated with adenomyosis prevalence (P = 0.05), and parturition gap length positively correlated with metritis prevalence (P = 0.033). Babirusa appear to be at highest risk for reproductive tract lesions. These data suggest that risk factors for reproductive tract lesions in Suidae include advanced age, prolonged gaps between pregnancies, and contraception.

Key words: Contraception, parity, pathology, reproductive tract, Suidae, Tayassuidae

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LITERATURE CITED


AMYLOID IN CAPTIVE BONGO (Tragelaphus eurycerus): IMPACTS ON MORBIDITY AND MORTALITY AND EVALUATION OF SERUM ACUTE PHASE PROTEINS PREMORTEM

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Abstract

A retrospective analysis was performed of histologic results from 96 deceased bongo (Tragelaphus eurycerus) submitted to Northwest ZooPath from 1995-2015. A high prevalence of amyloidosis was identified; 32% of animals had some degree of amyloid deposition in one or more organs. The degree of amyloidosis was clinically important in 58% of those cases, and assessed as the underlying cause of death in 42%. The most commonly affected organs were liver, kidney, adrenal, intestine, and rumen. Those most severely affected were pancreatic duct, ovary, liver, omasum, adrenal, and intestine. Fatal amyloidosis was commonly due to gastrointestinal complications.

To better understand the acute phase protein (APP) response in bongo and the high prevalence of amyloidosis, sera from 44 clinically normal bongo were tested by electrophoresis and for the APPs serum amyloid A and haptoglobin. Complete blood cell counts and fibrinogen levels also were analyzed when available. These values were compared to those from 13 abnormal animals (27 samples) with various infectious and/or inflammatory conditions. There was a statistically significant ($P \leq 0.05$) difference in levels of albumin (normal $2.73 \pm 0.09$ g/dl; abnormal $2.25 \pm 0.13$ g/dl), haptoglobin (normal $0.70 \pm 0.09$ mg/ml; abnormal $1.04 \pm 0.11$ mg/ml), and fibrinogen (normal $361 \pm 72$ mg/dl; abnormal $743 \pm 69$ mg/dl) between normal and abnormal animals. There was not a statistically significant difference in serum amyloid levels between normal (323.5 ± 92.1 mg/L) versus abnormal (566.9 ± 112.6 mg/L) animals. The high amyloid levels in both groups was unexpected. Further studies are needed.

Key words: Acute phase proteins, amyloid, bongo, mortality, Tragelaphus eurycerus
SUSPECTED MOXIDECTIN TOXICOSIS IN THREE SPECIES OF HOOFSTOCK AT A SEMI-FREE RANGE ZOOLOGICAL PARK

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Abstract

Moxidectin is a commonly used anti-parasitic in hoofstock that is distributed throughout the body and significantly lipophilic. It is labeled for use in cattle by oral, topical, and subcutaneous routes. In semi-free ranging conditions, many antiparasitics are administered intramuscularly via dart due to an inability to administer to an individual by other routes without anesthesia. During 2015-2016, three animals including a roan antelope (Hippotragus equinus), sable antelope (Hippotragus niger), and Arabian oryx (Oryx leucoryx) darted with moxidectin developed clinical signs consistent with toxicosis. The primary sign in all three cases was severe neurologic depression. Based on recommendations in canine cases, animals were treated with intravenous lipid therapy and supportive care while diagnostic testing was pending. All three initially improved prior to succumbing to secondary problems associated with prolonged recumbency. Moxidectin has been administered via dart on 73 occasions in seven different species at Fossil Rim during the past 3 yr, with only the above three cases showing clinical signs of toxicosis. Two potential causes in these cases include: poor body condition leading to excessive unbound drug in the bloodstream or a genetic defect similar to some herding dog breeds. Given that cases were seen in three different species at an overall low incidence within a given species, a genetic defect is considered unlikely. The animals affected did have significantly lower body condition scoring than their conspecifics, and it is considered likely that this predisposed these animals to toxicosis. Therefore, use caution when administering moxidectin via dart in animals in poor body condition.

Key words: Arabian oryx, Hippotragus equinus, Hippotragus niger, intravenous lipids, moxidectin, Oryx leucoryx, roan antelope, sable antelope, toxicosis

LITERATURE CITED


ALPHAHERPESVIRUS OUTBREAK ASSOCIATED WITH MORTALITY IN A
GROUP OF GREVY’S ZEBRA (Equus grevyi) HOUSED IN A MIXED-SPECIES
EXHIBIT: DIAGNOSIS, MANAGEMENT AND SURVEILLANCE

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Abstract

A herd of seven captive-born Grevy’s zebras (Equus grevyi) experienced a sudden outbreak of
nasal discharge and sneezing. Clinical signs were severe and acute in three animals and included
lethargy and anorexia. These animals were kept in separate stalls. A 16-mo-old zebra died within
48 hr of the onset of clinical signs. Three additional animals then developed mild nasal discharge
but no lethargy and one remained clinically asymptomatic.

Treatment of the remaining severely affected zebras (adult and 2-mo-old females) included
valacyclovir (40 mg/kg p.o., t.i.d.), meloxicam (0.6 mg/kg p.o./i.m.) and cefquinome (2.5 mg/kg
i.m. every 48 hr). The adult female improved rapidly and clinical signs resolved within 48 hr of
treatment; however, valacyclovir compliance was poor in the young female and rapid deterioration
and death occurred within 48 hr. The mildly affected animals were not treated and recovered
spontaneously.

Common findings at necropsy included severe fibrinonecrotic interstitial pneumonia that in one
case was associated with vascular thrombosis. Herpesvirus was detected on both individuals (lung,
nasal swab) by nested PCR. Sequencing of the amplicons revealed a novel sequence with 99.5%
similarity with previously published equine herpesvirus-1 (EHV-1) and equine herpesvirus-9
(EHV-9) sequences.

The zebras share housing facilities with other species, including white rhinoceros, giraffe, and
several antelope species. None of these individuals showed clinical signs; however, nasal swabs
and blood samples for PCR were collected. All were negative for herpesvirus except for the
springboks, which were positive for springbok herpesvirus 1.

This report illustrates the risk of herpesvirus outbreak in zebras. The source of the infection
remains unclear and investigation is ongoing.

Key words: Equus grevyi, Grevy’s zebra, herpesvirus, mixed-species, valacyclovir
LITERATURE CITED


SILICONE MATRIX EPISCLERAL CYCLOSPORINE IMPLANT FOR TREATMENT OF CORNEAL DISEASE IN MALAYAN TAPIR (Tapirus indicus)

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Abstract

Corneal disease and photokeratitis have been seen in certain species exposed to significant amounts of ultraviolet (UV) light.¹ For pinnipeds, inadequate shade was found to increase the likelihood of ocular disease by ten-fold.² Episceral cyclosporine subconjunctival implants have had positive clinical outcomes for treatment of otariid keratopathy for months to years (Colitz, unpubl. data). With their predominantly nocturnal and forest dwelling lifestyle, Malayan tapirs do not naturally have significant direct UV light exposure. In captivity, diurnal husbandry protocols, less dense forest canopy, and tropical UV light exposure have lead to corneal thickening, edema, and keratitis in two Malayan tapirs (Tapirus indicus) housed at Zoo Miami. The corneas exhibited waxing and waning stromal abscesses and keratitis, which were treated with multiple topical antimicrobial therapies. To limit the inflammatory process, a silicone matrix cyclosporine implant was used (North Carolina State University Veterinary Hospital pharmacy). Under general anesthesia, a surgical approach was made to the dorsal subconjunctival space and the implant was placed in the prepared tunnel and sutured in place. Comparative images 13 wk postoperatively showed decreased inflammation, neovascularization, and corneal edema. The manufacturer suggests that clinical effects can be seen for up to 8 mo; however, clinical results have been seen for longer in marine mammal species (Karpinski, personal communication). This case report describes the use of an immunomodulatory medication (cyclosporine) in a silicone matrix placed subconjunctivally for prolonged treatment of photokeratitis in Malayan tapir.

Key words: Cornea, cyclosporine, photokeratitis, photosensitivity, tapir, Tapirus indicus

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LITERATURE CITED


SYSTEMIC AMYLOIDOSIS IN A POPULATION OF PRONGHORN ANTELOPE
(Antilocapra americana)

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Abstract

Fourteen pronghorn antelope (Antilocapra americana) underwent complete or partial autopsies between 1997 and 2016. The animals ranged from 0.4-yr-old (5-mo-old) to 14.2-yr-old (7 ± 4.9); there were nine males, and five females. Ten pronghorn antelope had histologic evidence of amyloidosis resulting in a 71% prevalence of amyloidosis in the Columbus Zoo pronghorn antelope herd. The amyloid was further subtyped in two cases via mass spectrometry, with serum amyloid A and fibronectin detected. Serum amyloid A, beta and gamma globulin levels were evaluated and all were within normal ranges for healthy domestic cattle. Commonalities noted between most of the cases included historical and recent elevated fecal strongyle counts (Haemonchus spp.), repeated treatment for Haemonchus, anemia, hypoproteinemia and, in many cases, azotemia. Chronic inflammation due to pneumonia or endoparasitism was a predisposing risk factor to systemic amyloidosis in the pronghorn antelope herd. Given the possibility of a hereditary predisposition to amyloidosis, pedigree analysis was performed and there was no statistically significant difference between the mean degree of relatedness and amyloidosis. The most likely cause of the systemic amyloidosis was chronic inflammation caused by haemonchosis and/or pneumonia.

Key words: Amyloidosis, Antilocapra americana, Haemonchus contortus, hereditary, pronghorn

LITERATURE CITED

EFFECTS OF A HIGH-PHYTOESTROGEN DIET ON EQUINE ESTROUS CYCLES AND FERTILITY USING DOMESTIC MARES *(Equus caballus)* AS MODELS FOR WILD UNGULATES

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Abstract

Phytoestrogens are plant-based estrogenic compounds commonly found in zoological and domestic animal feeds composed of alfalfa, soy, and clover.4,6 Dietary phytoestrogens may play various roles in the infertility of several ungulate species.1-3,5,7 The reproductive health of domestic equids *(Equus caballus)* fed a commercial soy- and alfalfa-based exotic herbivore diet was investigated.a Twelve mares were fed the treatment diet February-November 2015 (T1) and subsequently fed a control diet February-November 2016 (C1). Mares were examined via transrectal palpation and ultrasound for estrous cycle and reproductive tract evaluations, and blood samples were collected weekly to obtain serum progesterone and estradiol concentrations. Toward the end of each trial, mares were artificially inseminated to evaluate fertility. It was hypothesized that T1 mares would exhibit abnormal cycle and phase lengths, lower progesterone values, higher estradiol values, and reduced conception rates compared to C1 mares. Results showed that T1 mares experienced significantly more short luteal phases (*P* = 0.015), higher average estradiol (*P* < 0.01), and lower average progesterone preceding the expected period of luteolysis (nonoverlapping 95% CI of trendlines). T1 mares also tended to exhibit fewer long cycles (*P* = 0.075) and more incidences of abnormal cervical tone and uterine edema during the expected luteal phase (*P* = 0.061). No difference in conception rates was found (*P* = 0.946). These results suggest that phytoestrogen-rich diets may alter key reproductive hormone concentrations and influence estrous cycle dynamics yet have no apparent effect on conception rates in horses. Further investigation into long term, gestational, and developmental exposure to phytoestrogens is necessary to better understand their relationship to equine fertility.

aMazuri® ADF-16 Herbivore Pellets, Purina Mills LLC, Gray Summit, Missouri 63039 USA

Key words: Diet, equine, estrous cycles, fertility, phytoestrogens, reproduction

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LITERATURE CITED


HUMAN MEDICAL EXPERIENCE PROVIDES PARADIGMS RELEVANT TO CAPTIVE BREEDING OF ENDANGERED WILDLIFE: RATIONALE FOR PREVENTION AND THERAPY OF HEMOLYTIC AND IRON OVERLOAD PROPENSITIES IN BROWSER RHINOCEROSES, TAPIRS, AND OTHER SUSCEPTIBLE SPECIES

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Abstract

Throughout history, advances in human medicine have relied heavily on experimental studies of animals. Conversely, insights into maladies affecting a wide range of animal species may be gleaned from the established pathophysiology of clinically similar syndromes in humans. Here examples are presented that demonstrate the value of this approach as well as potential hazards in its disregard. In the 1980s and 1990s, lethal hemolytic anemia often occurred among captive African black rhinoceroses (*Diceros bicornis*) following exposure to certain drugs or chemicals. These episodes resembled common disorders in humans caused by impaired ability to neutralize ambient oxidants. Comparative studies revealed metabolic characteristics of rhinoceros red blood cells (RBCs) that deviated radically from all other mammals, including a dearth (2-5%) of the essential metabolic fuel, adenosine triphosphate (ATP). Strategies to preserve and enhance RBC ATP, including avoidance of oxidant stresses and introduction of high-phosphate diets, were derived from human experiences and applied to rhinoceroses with consequent reductions in hemolytic episodes. Induction of hyperphosphatemia was additionally found to be an effective therapeutic intervention capable of interdicting active hemolysis. Iron storage disease (ISD) (aka iron overload disorder, IOD) is a progressive, clinically silent, multisystem disorder of high morbidity and mortality acquired by all browser rhinoceroses, tapirs and many other species of exotic wildlife when they are displaced from the natural environments in which they evolved. Recent demise of the captive breeding program for Sumatran rhinoceroses (*Dicerorhinus sumatrensis*) can be directly attributed to ISD. Extensive experience with hereditary and acquired ISD in humans provides strategies for prevention and therapy, but few institutions have adopted them despite their demonstrable effectiveness. Unlike their human analogs, these conditions in affected animals are not caused by aberrant genetic mutations but instead represent species-wide characteristics with important evolutionary implications.

**Key words:** African black rhinoceros (*Diceros bicornis*), hemolytic anemia, iron overload, phosphate, repetitive phlebotomy, Sumatran rhinoceros (*Dicerorhinus sumatrensis*)

**INTRODUCTION**

Animals have been studied since Antiquity for the benefit of mankind. Aristotle performed experiments with living animals, and fundamentals of anatomy, physiology and pathology established by Galen during the second century were based on his extensive animal dissections and observations. In more recent times, efficacy and safety of everything from cosmetics to drugs
to surgical procedures have crucially relied on animal testing, so it seems entirely appropriate that lessons learned from human studies should contribute equitably back to the health and welfare of animals. Notable current and historical examples are considered here, of a hemolytic syndrome affecting captive African black rhinoceroses (*Diceros bicornis*) and iron storage disease (ISD) or iron overload disorder (IOD) affecting all black and Sumatran (*Dicerorhinus sumatrensis*) rhinoceroses so far appropriately studied in captivity.

**ACUTE EPISODIC HEMOLYTIC ANEMIA**

Three decades ago, acute hemolytic anemia was the leading cause of death among captive black rhinoceroses, prompting intensive investigations into potential etiologies. Common causes of premature hemolysis, such as hemoglobin abnormalities and autoimmunity, were eliminated, but collaborative studies at the UCLA Hematology Research Laboratory revealed extraordinary disparities in biochemical and enzymatic characteristics between red blood cells (RBC) of all four rhinoceros species and other known mammals. These differences included significantly impaired capacities to neutralize reactive oxygen species (ROS) generated by ambient physiological and pathological processes. The discovery of minimal quantities (2-5%) of the essential high-energy compound, adenosine triphosphate (ATP) in rhinoceros RBCs, remains virtually unprecedented in vertebrate hematology. As the obligate fuel for cation pumping, ATP is essential for maintaining sodium/potassium gradients across cell membranes. This minimal reserve was viewed as the potential Achilles heel for erythrocyte viability, since failure of the cation pump allows influx of water, RBC expansion, and eventual lysis. Indeed, insufficient ATP is classically (but arguably) viewed by many as the terminal event in limiting normal RBC life spans and in inducing premature hemolysis in hereditary defects of erythrocyte metabolism.

The clinical phenotype of hemolytic anemia in black rhinoceroses is virtually identical to an analogous, highly common disorder in humans, glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. Based on extensive experience with such patients, preventive strategies for rhinoceroses are proposed, including avoidance of drugs, chemicals, foodstuffs and other conditions, such as acidosis and hypophosphatemia, that are known to initiate hemolysis in sensitive subjects. High-phosphate diets were additionally recommended because RBC ATP concentrations are well known to correlate closely with serum phosphate levels in humans. ATP concentrations also increase rapidly in rhinoceros RBCs incubated in phosphate media, with consequent decreases in their sensitivity to oxidant challenge. As a preventive measure, phosphate supplementation has been credited with virtual disappearance of acute hemolytic anemia over the past two decades and has been adopted by many (but not all) rhino-holding institutions. Nonetheless, another recent episode of acute hemolysis after drug administration to a black rhinoceros with marginal hypophosphatemia serves as a potent reminder that apparent success can breed complacency.

Data are presented supporting the value of dietary phosphate supplementation in black rhinos as a routine preventive measure as well as the potential use of parenteral phosphate infusions to interdict active hemolytic crises. In 1992-1993, three clinical cases provided opportunities to extend theoretical preventive strategies into active therapeutics. Blood specimens from three female black rhinos, approximately 20, 30 and 40 yr in age, were referred to the UCLA Hematology Research Laboratory from two institutions to help evaluate conditions that included
severe pododermatitis, mucocutaneous ulcerations and multiple geriatric problems. All were overtly or marginally hypophosphatemic.

One rhinoceros was maintained on dietary phosphate supplementation and sampled serially over a 3-mo period to follow hematologic indices and blood chemistries. Red cell adenine nucleotide (ATP+ADP+AMP) concentrations, measured by four different assay techniques, rose progressively as hypophosphatemia was corrected, eventually reaching levels 4- to 5-fold higher than control means. The second rhinoceros began to hemolyze, losing two-thirds of its circulating red cells over a 3-wk period. Multiple intravenous infusions of sodium phosphates were accompanied by tripling of total red cell adenine nucleotides and cessation of hemolysis. The third rhinoceros suddenly hemolyzed with gross hematuria and rapid loss of two-thirds of its circulating red cell mass. The animal was immobilized at frequent intervals to allow multiple infusions of parenteral phosphate. These were associated with rapid elevations in red cell ATP, cessation of hemolysis, erythroid regeneration and gradual return of packed cell volumes from a nadir of 16% to 45-48% (Figure 1).

These quantitative data substantiate conclusions drawn from in vitro studies regarding the importance of avoiding and/or correcting hypophosphatemia and inducing hyperphosphatemia to mitigate hemolytic tendencies in black rhinos. A recently identified mutation in the SLC28a2 gene of black (but not white) rhinoceroses likely contributes to their inherently low ATP reserves by affecting adenosine transport,\(^7\,30\) a crucial nucleotide salvage pathway for mammalian erythrocytes.\(^33\) Since ATP generation is also critically dependent on glucose catabolism, avoidance of other conditions that inhibit glycolysis, such as acidosis, is an equally important preventive measure. These observations additionally support the potential value of parenteral phosphate infusions to interdict episodes of active hemolysis.

**IRON STORAGE DISEASE OR IRON OVERLOAD DISORDER**

**Background**

Iron storage disease (ISD) is an inexcorably progressive, multisystem disorder that is typically devoid of clinical signs or symptoms until affected organ systems falter or fail. Both genetic (hereditary hemochromatosis) and acquired (transfusional) forms of ISD occur with very high frequency in humans, providing an enormous body of information regarding pathogenesis, diagnosis, treatment and prevention that can be beneficially extended to animals.\(^1\,35\)

Necropsy records of virtually every African black and Sumatran rhinoceros in captivity over the past six decades cite the presence of widespread and varying degrees of hemosiderosis, the morphologic, but not necessarily pathologic, hallmark of iron deposition. In many instances, because of the high incidence of hemolytic anemia, this was erroneously interpreted as residua of previous RBC lytic episodes. Necropsy observations with iron-specific stains and quantitative tissue analyses, provide unequivocal evidence of underlying pathophysiology involving iron homeostasis, resulting in clinically significant, sometimes massive, body burdens of highly toxic ferric iron.\(^16\,17\,21\,26-29\)
Due to species-wide genetic predispositions, all browser (but not grazer) rhinoceroses, tapirs, and many other genera of exotic wildlife, are in jeopardy of developing ISD when displaced from natural environments where they likely evolved dependence on environmental factors or crucial dietary components to reduce iron absorption and maintain iron balance. Within weeks, excess iron loads are detectable (by serum analyte or necropsy studies) in newborns or newly captives, and these increase logarithmically, reaching tenfold elevations in as little as 3-5 yr, more rapidly in Sumatran than in black rhinoceroses.

While precise causes of ISD in some species may be uncertain, no controversy exists about its consequences. Iron in excess is invariably deleterious to biologic systems because it catalytically generates highly toxic, hydroxyl free radicals and other ROS. Rhinoceros species are particularly vulnerable to ISD due to their inherently impaired capacities to neutralize ambient oxidants that are inevitable byproducts of aerobic metabolism. The toxicity of excess iron is also a likely cause or contributor to many of the disparate disorders acquired by browser rhinoceroses under captive conditions.

**Diagnosis**

Measurements of serum ferritin concentrations and transferrin saturation (the ratio of serum iron to total iron binding capacity, TIBC) provide the least invasive means to assess iron status. It is widely acknowledged that serum ferritin concentrations reflect total-body iron stores with an accuracy exceeded only by direct quantitative analyses of tissue samples. There are important caveats: ferritin is also an acute phase reactant produced in response to inflammatory stimuli, so other clinical conditions can confound interpretation of assay results. Additionally, there can be no valid reference ranges for “normality” when an entire population is affected, as is the case for captivity-induced ISD in black and Sumatran rhinoceroses. Free-ranging rhinoceroses provide the best comparative standards.

Most rhinoceros studies have relied on species-specific ferritin assay systems developed by Smith, et al, available through the Kansas State University Veterinary Diagnostic Laboratory. In separate studies, serum ferritin values measured by this assay in African black and white rhinoceroses free-ranging in their natural habitats were < 100 to 200 ng/ml and < 100 to ~350 ng/ml. By contrast, the mean ferritin concentration of 70 adult black rhinoceroses in U.S. captivity was 7,160 ng/ml, with individual values ranging > 10,000 to > 100,000 ng/ml. Such extreme elevations are far in excess of known synthetic rates for apoferritin, thereby providing direct evidence of organ damage sufficient to release intracellular ferritin. Also by contrast, specimens from 14 captive Sumatran rhinoceroses averaged > 850 ng/ml, with individual values ranging as high as 2,000-4,000 ng/ml and transferrin saturations of 90-100%, clearly indicative of significant iron overloads developing in captivity.

Recently, alternative systems for measuring ferritin and assessing ISD status have been proposed, but these have not yet been validated by direct comparisons or necropsy studies. Ferritin concentrations alone may not be diagnostic of ISD, but they provide an invaluable metric of total-body iron stores when properly interpreted in context with other data, such as transferrin saturation and the ultimate determinant, histopathology with iron-specific stains.
Transferrin saturation, the amount of iron bound to the plasma transport-protein transferrin, provides a simple, qualitatively reliable supplement or alternative if ferritin assays are equivocal or unavailable. Transferrin saturation correlates well with ferritin concentrations, with quantitative tissue analyses, and with histopathology using ferric-specific stains such as Prussian blue.\textsuperscript{16,21,27} Transferrin saturation in most vertebrates is \textasciitilde35\%. Values > 65-70\% are considered threshold for onset of overt multisystem organ pathology.\textsuperscript{1,35} U.S. captive Sumatrans measure 90-100\%, clearly indicating iron in sufficient excess to overwhelm carrying capacity of protective proteins.\textsuperscript{16,21,27,28}

**Prevention and Therapy**

Experiences over the past century with literally millions of humans with hereditary and acquired forms of ISD provide a strong foundation for extrapolation to animals. Patients with hereditary hemochromatosis typically remain asymptomatic until their fourth or fifth decades of life. If untreated, protean clinical signs and symptoms then begin to develop, reflecting endocrine, cardiac and/or hepatic dysfunction, eventually terminating in organ failure, cirrhosis and liver carcinomas by the age of sixty. With clinical intervention, the morbidity and mortality of progressive organ dysfunction can be entirely avoided, and shortened (25-35\%) life spans restored to normal, by periodically removing small aliquots of venous blood. This induces a slight anemia that signals bone marrow erythroblasts to mobilize iron stores for new RBC production, thereby averting excessive iron accumulation.

Phlebotomy protocols applicable to rhinoceroses and tapirs have been proposed\textsuperscript{18-20} and effectively applied to black rhinoceroses.\textsuperscript{9,10,12,38} It should be emphasized that this procedure is preventive (not therapeutic), and it is optimally suited for young and newly captured animals before they develop inordinate body burdens of iron. Since phlebotomy removes only \textasciitilde0.5 gram of hemoglobin iron per liter of blood, it cannot significantly reduce body burdens that reach kilogram amounts, as commonly occurs in long-term captive black and Sumatran rhinoceroses. As a therapeutic alternative, reduction of excess iron stores by pharmacologic chelation remains feasible, but prohibitively expensive. Phlebotomy is contraindicated in the presence of clinical or laboratory signs of anemia or organ dysfunction, since it would only compound those problems by inducing or accentuating anemia.

Despite their demonstrable effectiveness, phlebotomy programs have not yet been widely adopted by most rhino-holding institutions, largely because of the time and expense required to train and treat animals that superficially appear to be entirely healthy until organ dysfunction becomes overt and irreversible. This makes it difficult for responsible administrators to justify commitment of resources. That reluctance might diminish if cost/effect analyses considered the following: computer programs predict sustainability of animal populations in peril by assessing factors that affect birth/death ratios. Shifts as little as \pm 2-6\% may be capable of altering balances for certain rhinoceros populations. Based on vast experience with human ISD, preventive phlebotomy programs could increase life spans by 25-35\%, perhaps equivalent to two or three more breeding cycles that could be tipping points for effective captive-breeding programs.
Evolutionary Implications

The physiologic bases of these two detrimental conditions (impaired antioxidant metabolism and propensity to overload iron) share a common denominator: both represent species-wide characteristics rather than consequences of selective genetic mutations affecting only individuals or families. This raises the obvious question: what were the evolutionary pressures that promoted these apparently deleterious characteristics?

Again, human studies may provide some clues. The equatorial belt is dominated by populations with very high incidences of hemoglobinopathies and metabolic enzyme deficiencies. These disorders have persisted despite their high morbidity and mortality because they confer some degree of protection against the most malignant form of falciparum malaria. That selective advantage appears to be a result of lower levels of RBC ATP that characterize these conditions. Perhaps the dearth of ATP in rhinoceros RBCs provides similar protection against Babesia or other intraerythocytic parasites? This is a hypothesis that could be tested by quantitative assays for parasite infectivity in RBCs primed to varying levels of ATP by phosphate stimulation.

Disparities between browsers’ and grazers’ tendencies to overload iron also likely relate to their evolutionary origins. Since vertebrates lack the ability to excrete iron, appropriate concentrations of this critically essential element depend on modulation of enteric uptake. In the high-oxygen atmosphere of the Oligocene epoch when browsing mammals evolved, bioavailability of iron and other essential metals was very low, favoring physiologic mechanisms for avid uptake. When grasslands became ubiquitous during the subsequent Miocene, grazing mammals emerged, and their dietary specialization required additional adaptions to prevent excessive assimilation of soil iron. In the absence of similar mechanisms to limit uptake, it seems most likely that browsers prevent iron overloading by consuming forage components that form insoluble complexes with iron allowing its enteric passage. Natural chelators such as tannins, fiber, phytates, phenolics and other components are abundant in twigs, leaves, and bark of natural browse. L-mimosine, an amino acid with extremely high iron avidity, is concentrated in Mimosa species that are toxic to most animals but favored forage for rhinoceroses. The importance of dietary and environmental factors in iron homeostasis is emphasized by the observation that iron loading by Sumatran rhinoceroses residing in Southeast Asian sanctuaries, appears directly related to variations in their dietary and foraging tendencies. Without access to their natural forage, it’s not surprising that metabolic imbalances such as ISD are inevitable consequences for susceptible species.

SUMMARY AND CONCLUSIONS

The invaluable contributions that animal studies have made to the archives of human medicine may be partially reciprocated by application of the latter to the former. Studies of phenotypically similar human disorders have contributed successfully to amelioration of the hemolytic syndrome affecting black rhinoceroses in captivity. Attempts to apply similar strategies to ISD affecting multiple species of endangered wildlife have been far less effective largely because the insidious nature of ISD allows its progression devoid of overt clinical signs or symptoms, often until it results in terminal decline. Nonetheless, experience with equivalent human disorders strongly supports repetitive phlebotomies for prevention of ISD in susceptible wildlife species with the
strong probability that such programs would not only reduce morbidity and enhance quality of life, but would likely extend life spans and breeding cycles to the benefit of captive breeding programs.

ACKNOWLEDGMENTS

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LITERATURE CITED


Figure 1. Effect of parenteral phosphate infusions on RBC packed cell volumes, serum phosphate concentrations, and total RBC adenine nucleotide concentrations in a black rhinoceros during and following an acute hemolytic episode. Shaded bars indicate ranges of normal means ± 1 SD.
A MULTIFACETED PROGRAM FOR END-OF-LIFE CARE

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Abstract

Advances in husbandry, nutrition, and veterinary medicine have allowed improvements in geriatric animal management and enhanced end-of-life care in zoos and aquaria. Managing animals at the end of their lives can involve physical and social challenges. Challenges may also exist with difficult decision making and a diversity of staff and public opinion. Many facilities are seeking a better understanding of end-of-life care, while trying to improve communications with regards to such care to staff and visitors.

In 2013, Disney’s Animals, Science and Environment team initiated a program to improve care for aging animals and promote staff discussion around this topic. The initial effort focused on presenting a geriatric animal care seminar for animal caretakers.\textsuperscript{1} From that seminar, multidisciplinary subcommittees were formed to tackle four key objectives: 1) create a resource center, 2) focus on geriatric animal training, 3) create a quality-of-life assessment toolkit, and 4) develop training on end-of-life care. A Sharepoint resource center now allows staff to access materials on subjects from quality-of-life assessment to grief management. A focus group with representatives from all husbandry teams was formed, and holds quarterly forums to share best practices on care and training of geriatric animals. A quality-of-life assessment toolkit was developed and is now widely used to guide end-of-life discussions. Finally, a 4-hr training course was established, which focuses on assessing, communicating, and elevating the quality of life for aged animals; this course is now required training for all Disney animal care staff. These end-of-life care initiatives have resulted in improved communications, better decision-making processes, and ultimately, advancement of end-of-life animal care at Disney.

Key words: Communication, end-of-life care, geriatric animals, quality of life, toolkit, training course

ACKNOWLEDGMENTS

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LITERATURE CITED

ADDITION OF COMPLEMENTARY THERAPIES TO ZOOLOGIC MEDICINE

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Abstract

Complementary therapies, including rehabilitation, Traditional Chinese Medicine (TCM), and chiropractic care are adjunct treatments that can be used in conjunction with more conventional therapies to treat a variety of medical conditions. Complementary therapies do not need to be alternatives to western medicine, but rather can be used simultaneously. The addition of complementary therapies to the practice at the Houston Zoo has enhanced the quality of life, longevity, and positive outcomes for various cases across multiple taxa.

Over the last 42 mo, the Houston Zoo has consulted with a veterinarian certified in chiropractic, acupuncture, and rehabilitation therapies (complementary therapy veterinarian), who has provided treatment to over 40 species. The zoo’s veterinarians communicate directly with the complementary therapy veterinarian for case selection and are present at each patient’s initial evaluation to discuss history, treatment feasibility, and to formulate a comprehensive treatment plan. The initial evaluation also includes feedback from the keepers and their supervisory staff about what the individual animal will tolerate and ongoing training behaviors that keepers can implement in order to facilitate future treatments. This arrangement has empowered keepers to have greater primary responsibility, to train for a specific therapy goal, and to actively participate in an animal’s medical care. Whenever possible, followup visits with patients usually are completed by the complementary therapy veterinarian independently. This allows more time to be spent with the patients during their therapy sessions, thereby allowing the zoo veterinarian to attend to the remainder of the zoo population.

Patients most commonly treated with complementary therapies are geriatric and have musculoskeletal weaknesses that can be addressed with creative rehabilitation techniques. For instance, a Komodo dragon (Varanus komodensis) with neck pain stemming from vertebral subluxations and weak cervical muscles was prescribed resistance¹ band exercises to strengthen those muscles. Another example involved a geriatric leopard (Panthera pardus) with cervical nerve root impingement causing a forelimb lameness. This animal was taught exercises to stretch out its neck using target training. Frequent training sessions for these exercises provided enrichment and improved the leopard’s condition. The exercises resulted in the leopard making fuller use of its exhibit space, including areas historically avoided due to its cervical instability.

Rehabilitation therapies also have been utilized to promote recovery of orthopedic injuries after surgical repair. As an example, elastic² adhesive tape and specific exercises were successfully used to strengthen and maintain maximum range of motion in a flamingo’s (Phoenicopterus chilensis) wing post ulna fracture repair. Other rehabilitation therapies used at the Houston Zoo
have included changing enclosure substrates to ease movement or encourage muscle development, adding exercise equipment (such as ground poles that must be stepped over when entering and exiting a barn), feeding in a narrow chute to encourage the animal to back out of it to build hamstring muscles, and adding produce to water features to encourage swimming retrieval of the food.

TCM encompasses many practices. The technique utilized most frequently at the Houston Zoo is acupuncture. There are 14 meridians, or acupuncture channels, that run throughout the body. Acupoints are located along these channels and when stimulated, work to regulate the body’s circulation and nerve function. The acupoints can be stimulated in a variety of ways, with the most common methods utilized at the Houston Zoo being dry needle acupuncture, laser acupuncture, and electroacupuncture. The selection of which type of stimulant for the acupoints for each patient is based on patient cooperation and safety. For example, a grizzly bear (*Ursus arctos*), received laser acupuncture through protected contact. Trained or conditioned animals that can have direct contact, such as the Komodo dragon, do well with dry needle acupuncture. Acupuncture therapy also has been utilized when patients are under anesthesia for routine or diagnostic procedures. For example, a female Western lowland gorilla (*Gorilla gorilla gorilla*) was anesthetized for a routine examination and the complementary therapy veterinarian was able to work in concert with the zoo veterinarians while other procedures were performed. Acupuncture needles were used to help with chronic hip dysplasia and to balance reproductive hormones without increasing the anesthesia time. Following its acupuncture treatment, the gorilla displayed breeding behavior for the first time at the Houston Zoo.

Chiropractic care aims to restore normal interactions between the spine and nervous system. A chiropractic adjustment is a precise movement at a specific angle with a controlled force and is used to remove chiropractic subluxations, which are adjacent joints lacking normal motion and/or alignment. Chiropractic care can be used on animals that are awake or under anesthesia. Patients that cannot be directly manipulated while awake may be adjusted while recovering from anesthesia for other procedures without increasing the total anesthesia time. At the Houston Zoo, the complementary therapy veterinarian has used chiropractic care to help with an array of cases including acute and chronic lameness in various species, self-mutilation of the tail in a leopard, and chronic neck pain in a chimpanzee (*Pan troglodytes*). Another example is a St. Vincent Amazon parrot (*Amazona guildingii*), that was unable to use its right pelvic limb at 1 mo of age. The cause of this functional deficiency was undetermined despite a thorough medical evaluation. Chiropractic adjustment of the pelvis and hip restored normal function to this limb and the chick proceeded to develop normally. This case is an example of complementary therapy providing improvement when western medicine did not.

In summary, complementary therapies at the Houston Zoo are beneficial to a wide variety of cases. The collaborative relationships of the complementary therapy veterinarian, zoo veterinarians, and keeper staff are the key to success. This discipline adds another tool to use, along with western medicine, to help improve overall patient quality of life.

*REP Band Level 3, Magister Corporation, Chattanooga, Tennessee 37405 USA*

*RockTape Canine kinesiology tape, RockTape USA, Campbell, California 95008 USA*
MODEL Pointer Pulse handheld pulsed Laser and pulsed TENS, Lhasa OMS, Inc, Weymouth, Massachusetts 02189 USA
Electronic Acupunctoscope WQ-6F, Chi Institute of Chinese Medicine Inc, Reddick, Florida USA

**Key words:** Acupuncture, alternative, chiropractic, complementary, rehabilitation

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CAREGIVER PLACEBO EFFECT ON ZOO-ANIMAL WELFARE: APPLICATION OF A PRESSURE WALKWAY SYSTEM FOR OBJECTIVE EVALUATION OF LAMENESS IN A ZOO SETTING

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Abstract

In domestic animals, force plates and pressure-sensitive walkways have been used to characterize normal and abnormal gait variables, yet this technology rarely has been applied in nondomestic animals. The use of gait analysis technology provides an objective assessment of analgesic efficacy and is a valuable tool for characterizing the impact of caregiver and veterinarian bias on lameness scoring in domestic animals. In pets, an owner or caregiver placebo effect can profoundly affect interpretation of analgesic efficacy and lead to false assumptions on alleviation of pain. Zoo clinicians often heavily depend on the interpretations of animal care staff to assess treatment efficacy, a scenario inherently prone to misinterpretations and bias. Furthermore, with medical advances, geriatric care of zoo animals with degenerative joint disease is increasingly prevalent. Biased assessments of analgesic efficacy can significantly impact quality of life and appropriate welfare decisions. Pressure-sensitive walkway technology provides an opportunity to objectively assess some of these welfare considerations. A 10-ft-long Tekscan® Walkway™ System was installed to objectively evaluate lameness concerns and analgesic efficacy in nondomestic animals. A custom-built housing for the system has facilitated use in a variety of species. Stance time, stride time, stride length, stride velocity, maximum force, and maximum peak pressure can be calculated to detect lameness-associated alterations in gait. In addition, static weight distribution across the plantar foot surface can be a useful tool for comparisons between limbs in animals in a stationary stance. Species-specific data from sound animals must be established, but with this in place, prospective research on the response to analgesic efficacy is possible. Normal values in Humboldt penguins have been reported as proof of concept and authors are expanding to additional species. These normal values have allowed detection and quantification of lameness and gait alterations in clinically abnormal specimens, followed by controlled objective studies on the efficacy of analgesic therapies.

Key words: Analgesia, animal welfare, force plate, gait analysis, lameness, placebo

LITERATURE CITED


ALLOSTATIC LOAD: QUANTIFYING CHRONIC AND LONG-TERM INTERMITTENT STRESS TO IMPROVE HEALTH AND WELL-BEING IN ANIMALS

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Abstract

“After decades of resistance, there is now a genuine consensus that disease cannot be prevented or even successfully treated unless the role of stress is addressed alongside traditionally recognized factors such as genes and the environment.”1

Practitioners of zoological medicine have long known that the stress of nonnative environments, unusual social groupings, human proximity, and space restriction can lead to immunosuppression and compromised health. To date, metrics for stress have been limited primarily to evaluation of circulating glucocorticoid levels in blood, saliva, urine and feces. In human medicine, the impacts of chronic stress on physiologic dysregulation, termed allostatic load, have been evaluated using a composite of biomarkers known as the allostatic load index (ALI). Measures of ALI are based on physiologic adaptation to stress which, when repeatedly or chronically stimulated, leads to systemic dysfunction and disease.1,2 In humans, ALI is calculated using a panel of biomarkers of cardiovascular, metabolic and immunologic health, and is associated with both numerous health disorders and increased risk of mortality.3 Recently, the ALI model has been adapted for nonhuman primates, and shows promise as a measure of chronic stress in these species. In Western lowland gorillas (Gorilla gorilla gorilla) in human care, for instance, ALI is directly related to age, sex, and stressful events,4 and wild-caught females demonstrate higher ALI than those mother-reared in zoologic institutions.5 Adaptation of ALI to wildlife species will require species-specific assay validation and ongoing biomarker evaluation, but promises to yield an invaluable tool for predicting and preventing stress-related disease and improving well-being in these treasured animals.

Key words: Allostasis, primate, stress measurement, well-being, wildlife

LITERATURE CITED


ENHANCING CONSERVATION THROUGH VETERINARY CARE OF THE WHITE-BELLED TREE PANGOLIN (*Manis tricuspis*)

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**Abstract**

Pangolin are the most globally trafficked mammal. The IUCN states the white-bellied or African tree pangolin (*Manis tricuspis*, also known as *Phataginus tricuspis*) is subject to widespread and intensive exploitation for meat and scales within Africa. Recent listing of all pangolin species on CITES Appendix I highlights the need for improved understanding of this poorly studied species. Information is lacking on basic husbandry, reproductive physiology, and veterinary care of this species. A population of 32 individuals was transitioned over the past year from native habitat to professional managed care at six American facilities to establish a sustainable breeding population. Care of these animals has allowed for the development of reference intervals for hematology, serum biochemistry, trace elements and urinalysis. Parasitism, gastrointestinal disease, and nutritional challenges were significant life threatening conditions in this population. A novel diet has been well accepted, but animals are prone to anorexia and hyporexia associated enteritis and gastric ulceration. Nutritional support using tube feeding of commercially available formula was successful for nutritional stabilization. Ten females were pregnant at arrival. Pregnancy was monitored (*n* = 7) through ultrasound and radiographic imaging. Five individuals successfully gave birth to healthy offspring. Two pregnancies ended with stillbirth. One dam died during pregnancy. Dystocia in two animals was treated by caesarian section. *Clostridium perfringens* (*n* = 1) and inanition (*n* = 1) were associated with acute death in two offspring during weaning. One offspring is still nursing and three are currently weaning. All four offspring are doing well.

**Key words:** *Manis tricuspis*, morbidity review, pangolin, *Phataginus tricuspis*
ORGANIZATIONAL INFLUENCE: WHAT ZOO LEADERS SAY ABOUT VETERINARIANS

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Abstract

Organizational influence is the power to shape policy and planning. It can be the catalyst for making the right things happen. When a zoo or aquarium veterinarian’s ability to influence others is high, it benefits the reputation of the person, their team, the organization, and the profession. Ultimately, of course, the purpose of growing organizational influence is to enhance animal health and welfare.

Key words: Organizational influence, veterinarian, zoo leaders

INTRODUCTION

To better understand the factors that may limit zoo veterinarians’ influence in zoos, interviews were conducted with leaders in AZA-accredited institutions. The author wanted to determine what actions veterinarians can take to grow their organizational influence. The selection of interviewees was based on factors including career impact as a leader and experience working with or as zoo veterinarians. The choice of those interviewed was not intended to be scientific nor representative of the zoo industry as a whole. The individuals selected were skewed towards those familiar to the author. Also, the interview dialogue and interpretations by the author were necessarily subjective and general. Thirty individuals were interviewed. They included zoo directors and CEOs, senior vice presidents for animal care, curators, animal health directors, other zoo veterinarian leaders, and recently board-certified zoo veterinarians. Each leader was asked three questions:

1. What holds zoo veterinarians back from having more influence in their organizations?
2. What special value should zoo veterinarians provide zoo organizations?
3. What can zoo veterinarians change about themselves to increase their organizational influence?

DISCUSSION

Several common themes emerged from the first question about obstacles to influence. The most common point was that zoo veterinarians tend to isolate themselves in their medical role. One leader stated, “Zoo veterinarians focus on their world of medicine and lack appreciation of the organization as a whole.” Another common response was that zoo veterinarians have a reputation for creating unnecessary obstacles to programs and plans. The perception is that many zoo veterinarians are risk averse to the extreme and become rigid in their approach. Also, some thought that veterinarians tend to act self-important and show off their degree. A director commented, “They use knowledge and intellect as a weapon showing no desire for partnership.” Another addressed the same concept by stating, “Veterinarians do not acknowledge that the organization sustaining animals must stay healthy itself.” One director gave an example of how it can be
different. That director chose to promote a veterinarian to the executive level. That veterinarian “made a radical change.” He explained, “A focused clinician became an executive looking at things from a far broader perspective. That person blossomed, showing great insight.”

The second question asked about what special value zoo veterinarians should provide their organizations but perhaps are not given the opportunity. The most common response was that zoo veterinarians should serve as animal care advocates. Zoo veterinarians are icons for animal health and welfare. This role as animal advocates is valuable for the reputation of the organization. One person put it well: “Veterinarians are just plain credible as a profession.” Another common response was about problem solving. Veterinarians, due to their training and daily experiences, have a reputation as evidence-based problem solvers. They are good at analyzing and providing sensible solutions to complex problems. Their positive societal reputation is likely based on having the combined traits of compassion and scientific objectivity. But this may not match the organizational reputation. One CEO explained the root of this disparity by stating, “Institutional management tends to focus on strategy while veterinary practice tends to focus on operational and individual cases.” Others commented on the value of zoo veterinarians in zoonoses management and epidemiology. The zoo veterinarian has a working understanding of the One Health concept. As a CEO stated, “Zoo veterinarians are in a position to protect us.”

The responses to the third question followed a similar theme. For a veterinarian to gain influence, having a holistic approach was what most suggested. Zoo veterinarians should focus on the health of the whole organization rather than on themselves or the animal health role only. One CEO stated, “Learn to step away from being a zoo veterinarian and become more interested in people.” To accomplish this, another suggested that zoo veterinarians look for leadership opportunities in their organization outside of veterinary medicine. A leader summarized this idea by saying “Listen, learn, and understand the rest of the business. Don’t allow yourself to be isolated to clinical medicine alone.” Another typical response to this question was about learning to listen and improving self-awareness. Several of the interviewees thought that it was important to shift focus from self and move towards serving the needs of others. Some mentioned that zoo veterinarians should have a more realistic approach to risk. Addressing this point, a zoo operations executive was blunt. “Don’t be afraid to disagree, but don’t disagree just to disagree. Don’t go to the worst case just to get attention on the matter.” Recent efforts by zoo veterinarians to perform disease risk assessments and risk-based quarantines are positive examples of how to approach risk.

**SUMMARY**

In summary, here are three general observations to consider in creating a plan to gain organizational influence:

1. Veterinarians tend to have a reputation among zoo leaders for having a narrow interest and for creating obstacles to plans and programs. Opportunities exist to change this perception of zoo veterinarians.

2. Zoo leaders see veterinarians’ unique value as organizational icons for protecting animal health and welfare due to their societal reputation for objectivity and compassion.
3. Zoo leaders expect their senior veterinarians, like other senior leaders, to show interest in the whole organization and its people, not just their field of animal health.

To the latter point, one zoo veterinarian executive said, “It’s your zoo, and you want it to be successful. It always comes down to animal welfare, not about being right or wrong.”

The zoo veterinary profession has an opportunity to use its unique value and societal reputation to enhance its reputation among zoo leadership. By doing so, zoo veterinarians can grow their organizational influence and have a positive impact on organizational strategy and vision. Ultimately, that influence will help enhance animal health and welfare.
DIRECT INSPIRATION: ZOO VETERINARY COMMUNITY OUTREACH OPPORTUNITIES

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Abstract

Veterinarians tend to think of their role in the zoo as supporting inspiration and education of the public by keeping the collection healthy and happy. There are opportunities for zoo veterinarians to engage directly with the public and the local community. Opportunities may vary with each institution and location and may include high school career exploration programs, community science, technology, engineering, and mathematics (STEM) events, pre-veterinary club events, and one-on-one mentorship programs. Recently a unique educational offering was created at the Wildlife Conservation Society’s parks: the Little Zoo Vets program. Classes designed for third through fifth graders have been developed using a combination of images, videos, sounds, actual veterinary equipment, live animals, and custom-built models. Classes are taught by a zoo veterinarian with assistance from other hospital staff. The program has been offered in an after-school format for a series of eight classes over a semester and in an after-camp format as a series of five classes within a week. Topics include physical exam, blood tests, fecal exams, anesthesia, and darting, among others. Students are invited to participate in other education offerings at the zoo and parents are invited to become supporters. Innovative outreach programs such as Little Zoo Vets can enable veterinarians to contribute directly to their institution’s educational mission, reputation, and potentially revenue, all while being highly rewarding and fun to execute!

Key words: Community, outreach, public
DENTAL DISEASE AND A LEFT DISPLACEMENT OF THE ABOMASUM IN A RETICULATED GIRAFFE (Giraffa camelopardalis reticulate): LESSONS LEARNED

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Abstract

A 10-yr-old reticulated giraffe (Giraffa camelopardalis reticulate) bull presented with acute abdominal pain (colic). The bull had a 4-mo history of being a poor eater. Physical examination, management, and monitoring over the following 72 hr were performed with the aid of two standing sedations. The giraffe developed a metabolic alkalosis and pre-renal azotemia. A metallic ping sound was auscultated on the left side near the 10-12th rib. Administration of intravenous fluids via jugular catheter was unsuccessful due to the high venous blood pressure. Pre-renal azotemia worsened and compensatory respiratory acidosis and paradoxical aciduria developed. Due to worsening condition and inability to stabilize the giraffe, euthanasia was elected. On necropsy there was a left displaced abomasum (LDA) with fluid sequestration (40-50 gallons) within the rumen. The molars had flattening of the occlusal surfaces and a computer tomography scan of the head showed significant dental disease. This case reinforces that giraffe considered poor eaters should be evaluated for dental disease. A high-concentrate low-roughage diet, reflective of how giraffes are fed in captivity, is the main predisposing factor for LDA development in periparturient dairy cows. During necropsy the position of the abomasum must be noted prior to removal of the gastro-intestinal tract to ensure that cases of LDA are not missed. This case emphasizes the importance of blood gas analysis as a diagnostic tool for ruminants with acute abdomen. Giraffe critical care poses many unique challenges and each case adds vital information to future veterinary management of this species.

Key words: Dental disease, Giraffa camelopardalis reticulate, giraffe, left displaced abomasum, metabolic alkalosis

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LITERATURE CITED


GENERAL ANESTHESIA AND SURGERY TO TREAT A MANDIBULAR SEQUESTRUM IN AN ATLANTIC BOTTLENOSE DOLPHIN (*Tursiops truncatus*)

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Abstract

An adult male bottlenose dolphin (*Tursiops truncatus*) presented with diffuse swelling of the frenulum and intermittent swelling along the lateral aspect of the right mandible. This dolphin had sustained an open fracture of the right caudal mandible 6 yr previously. Radiographs revealed chronic sequestrum formation with free fragments of bone present within a well demarcated lytic cavity. Treatment of the sequestrum required general anesthesia and surgical debridement of the mandible. The dolphin received 0.27 mg/kg oral diazepam in a single easily digestible fish 2 hr prior to handling. Additional pre-medications (0.05 mg/kg midazolam and 0.1 mg/kg butorphanol i.m.) were administered immediately after removing dolphin from pool. For induction, 5 mg midazolam, then 3 mg/kg propofol were administered slowly through an i.v. catheter placed in the lateral peduncle. Intubation was accomplished by manual dislocation of the goosebeak and insertion of 18-mm ET tube. Anesthetic maintenance was with sevoflurane at 1-3%. Surgery was performed with the dolphin in sternal recumbency to allow for optimal ventilation. An incision was made on the lateral aspect of the mandible after review of pertinent vasculature.¹ Bone curettes were used to remove any abnormal tissue, then endogenous PRP was infused into the bone defect. Closure was in two layers with overlying silicone button stents to help distribute tension over skin. Flumazenil, naloxone, and then naltrexone were all given intravenously to reverse sedative and respiratory depressant effects of midazolam and butorphanol. The dolphin recovered well from anesthesia and surgery and exhibited decreased swelling and improved comfort (interpreted by cessation in rubbing behavior) following the procedure.

Key words: General anesthesia, mandible, sequestrum, surgery, *Tursiops truncatus*

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The authors would like to thank the Veterinary Services and Animal Care Department at SeaWorld Orlando for their dedication and assistance in this dolphin’s care.

LITERATURE CITED

LONG-TERM MANAGEMENT OF FUNGAL PNEUMONIA (Coccidioides immitis) IN A KIRK’S DIK-DIK (Madoqua kirkii) IN A HIGHLY ENDEMIC AREA

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Abstract

An 8-yr-old female Kirk’s dik-dik (Madoqua kirkii) housed in a southwestern United States zoo was anesthetized for examination due to declining body condition, hyporexia, poor hair coat, lethargy, and abnormal behavior of shivering and defecating in atypical locations. The only abnormalities noted on physical examination were thin body condition and prominent lung sounds on auscultation. Thoracic radiographs showed a mixed alveolar and structured interstitial pattern throughout all lung lobes. Complete blood cell count and serum chemistry panel revealed a severe leukocytosis due to neutrophilia with a left shift, severe normocytic-normochromic anemia, and severe hyperglobulinemia. Due to the high incidence of coccidioidomycosis in the southwestern United States, all mammalian chemistry samples from this zoological institution are assessed for a coccidiodomycosis titer. This animal was found to have a severely elevated coccidioidomycosis titer of > 1:256 with the presence of both IgG and IgM antibodies, suggestive of active infection. Coccidioides immitis is a pathogenic dimorphic fungi, also known as valley fever. While numerous other taxa (including great apes, prosimians, old and new world primates, equids, mustelids, and procyonids) have been diagnosed with and treated for coccidioidomycosis, no members of the subfamily Antilopinae have been reported as infected to date. Oral fluconazole therapy was initiated at 5.3 mg/kg daily. Due to insufficient fluconazole levels (4.34 µg/ml) measured 1 mo into treatment, the dosage was increased to 10 mg/kg daily, which eventually resulted in therapeutic drug levels. To date, this dik-dik has been treated for 23 mo with resolution of clinical signs, and marked improvement in blood work and valley fever titer values. While uncommon in ruminants, coccidioidomycosis is an important differential for systemic disease and pulmonary abnormalities in exotic ruminants housed in southwestern United States institutions.

Key words: Coccidioides immitis, dik-dik, fungal pneumonia

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LITERATURE CITED


CARDIAC PACEMAKER IMPLANTATION FOR MANAGEMENT OF ATRIOVENTRICULAR BLOCK IN TWO TASMANIAN DEVILS (Sarcophilus harrisii)

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Abstract

Atrioventricular block (both second-degree and third-degree) has been documented in aged Tasmanian devils (Sarcophilus harrisii), with onset of disease typically at > 5 yr of age.¹ Recommended therapy consists of surgical implantation of a pacemaker to bypass abnormal conduction fibers and allow for appropriate ventricular contraction. Despite being considered a simple surgical procedure in many species, pacemaker implantation has not been performed in a Tasmanian devil. Two male Tasmanian devils, ages 3.5 and 6 yr, were diagnosed with atrioventricular block after acute onset of intermittent collapse and during a routine health examination, respectively. Electrocardiography revealed atrioventricular block in both cases. Radiography and echocardiography confirmed the absence of congestive heart failure. A unipolar ventricular cardiac pacemaker was placed via a trans-diaphragmatic approach, with the generator placed in a pocket created in the transversus abdominis muscle. Both animals recovered from surgery and have had no postoperative complications. Two recheck exams each have confirmed successful alleviation of clinical signs of conduction deficits with no loss of cardiac muscle contractility. Although not validated in Tasmanian devils, troponin I was measured to evaluate for myocardial damage. Compared to troponin I levels in three unaffected devils, troponin I levels in the devils with atrioventricular block were 10- to 37-fold higher at the time of pacemaker implantation. Troponin I levels then declined after pacemaker placement though at different rates. These two cases illustrate that cardiac pacemakers can be placed in Tasmanian devils, resulting in improved cardiac function and quality of life for affected animals.

Key words: Atrioventricular block, pacemaker, Sarcophilus harrisii, Tasmanian devil, troponin I

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LITERATURE CITED

CONGENITAL DILATED CARDIOMYOPATHY WITH CONGESTIVE HEART FAILURE IN A RHINOCEROS HORNBILL (Buceros rhinoceros)

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Abstract

A 3-mo-old rhinoceros hornbill (Buceros rhinoceros) presented for a distended coelom, wet cough and hock-sitting stance. The animal had a history of poor growth and clumsy flight attempts compared to other offspring of the same age. On examination the bird was found to have severe coelomic and pericardial effusion, and an approximately 2 cm diameter, round, left lateral coelomic body wall hernia. Two-dimensional (2D) echocardiography revealed severe left atrial and ventricular dilation with moderate right atrial and ventricular dilation. The contractility of both ventricles was severely reduced with a fractional shorting of 16% and an ejection fraction of 34%. Color Doppler flow imaging showed severe mitral and tricuspid regurgitation associated with dilation of the annulus of the mitral and tricuspid valves, and severe pulmonary hypertension. The bird was treated with daily furosemide and supplemental oxygen therapy; however, it arrested 4 days after initial presentation. On postmortem examination, significant pericardial and coelomic effusions were present, consistent with right-sided congestive heart failure. The heart was thin walled, diffusely enlarged, and histologically unremarkable, indicative of a congenital lesion. Severe, chronic, passive congestion of the lungs was also present, consistent with left-sided congestive heart failure. Imaging and consultation with a veterinary cardiologist was integral to antemortem diagnosis of this first reported case of congenital dilated cardiomyopathy in a hornbill species.

Key words: Buceros rhinoceros, congenital, dilated cardiomyopathy, hornbill

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A PRIMARY URINARY BLADDER TERATOMA IN A MANED WOLF (*Chrysocyon brachyurus*)

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**Abstract**

A captive-born, 5.5-yr-old female maned wolf (*Chrysocyon brachyurus*) was treated with antimicrobials for two bouts of stranguria due to suspected cystitis. Treatment did not result in resolution, so the animal was anesthetized for further evaluation. On physical examination, the bladder was extremely firm and abdominal ultrasound showed marked thickening of the urinary bladder wall with no observable lumen. Neoplastic invasion of the bladder was suspected on double contrast cystogram and confirmed by surgical exploration. As the mass encompassed most of the bladder, it was considered inoperable and the wolf was euthanized.

Gross necropsy revealed a firm, white mass involving 80% of the luminal bladder surface, and a cerebriform mass extending into the bladder lumen. Histopathologic examination of the bladder and associated masses revealed a neoplasm comprised of multiple tissue types, including chondroid, bone, neural, and ring cells, as well as varying epithelial forms. Other features included multifocal necrosis and vascular invasion. The bladder mass was diagnosed as a malignant extragonadal teratoma.

Few extragonadal teratomas have been described in animal species. This is the first reported case of a teratoma originating at the urinary bladder in any nonhuman species.

**Key words:** Bladder neoplasia, *Chrysocyon brachyurus*, double contrast cystogram, maned wolf, stranguria, teratoma

**ACKNOWLEDGMENTS**

The authors wish to thank the animal care staff of the Endangered Wolf Center and the staff of the Saint Louis Zoo’s Department of Animal Health for their care and dedication to this animal.
DIAGNOSIS AND TREATMENT OF HYPERTHYROIDISM IN A GUANACO (Lama guanicoe)

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Abstract

This is the first reported diagnosis and treatment of hyperthyroidism in a camelid. A guanaco (Lama guanicoe) is a nondomestic relative of the llama (Lama glama). Guanacos are native to the mountainous regions of South America, have entered the United States through the pet trade, and are popular exhibit animals. A privately owned guanaco presented to the Veterinary Health Center at Kansas State University for a swelling located on the proximal, ventral neck. Ultrasound and cytology revealed a cyst-like structure with proteinaceous fluid, macrophagic inflammation and epithelial proliferation. A diagnosis of hyperplastic or neoplastic parenchyma was proposed. The initial total thyroxine (T4) and triiodothyronine (T3) results were 176 nmol/L and > 4.7 nmol/L, respectively. Patient T4 and T3 levels were subsequently run in sequence with a healthy companion (171 nmol/L, > 4.7 nmol/L; 106 nmol/L, 1.1 nmol/L) and the patient’s thyroid levels were considered elevated. Nuclear scintigraphy was performed and the radiopharmaceutical uptake indicated hyperactive tissue in the lateral margins of the left and right thyroid gland. Treatment included a subcutaneous injection of radioactive iodine therapy (I131) at a dose of 50 mCi. Two and 8-wk recheck exams showed declining T4 levels, but were maintained within camelid reference ranges. Fourteen months post I131 treatment the patient experienced a hypothyroid event (T4: 9 nmol/L, T3: 0 nmol/L). Levothyroxine was initiated at 0.06 mg/kg s.i.d. for 1 mo. At a 4-mo recheck the patient was clinically healthy, the swelling had regressed and T4 levels were consistent with those post I131 treatment.

Key words: Camelid, guanaco, hyperthyroidism, Lama guanicoe, radioactive iodine therapy (I131), thyroxine

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LITERATURE CITED

EVALUATION OF AMANTADINE IN A MULTIMODAL ANALGESIC REGIMEN FOR ALLEVIATION OF OSTEOPATHRITIS IN MULTIPLE CAPTIVE ZOO SPECIES

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Abstract

A variety of captive zoo species with chronic pain from osteoarthritis were prescribed amantadine hydrochloride as part of a multimodal pain therapy. Animals that received this drug included an adult female California sea lion (Zalophus californianus), an adult male hybrid orangutan (Pongo spp.), two adult female rockhopper penguins (Eudyptes chrysocome), an adult Kangal dog (Canis lupus familiaris kangal), and an adult female American bald eagle (Haliaeetus leucocephalus). Each animal had a history of decreased mobility and activity, and some had decreased appetite and interaction with trainers and/or other animals in the enclosure. Previous radiographs confirmed osteoarthritis in various joints of each animal. Nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and gabapentin had previously been prescribed to the animals; however, recurrent signs of pain were observed even at higher dosages of each medication. The N-methyl-D-aspartate receptor (NDMA) antagonist, amantadine, was trialed with each animal at dosages ranging from 1.1-3 mg/kg p.o., s.i.d. Prior to and after administration of amantadine, animals were assessed for pain and mobility and scored for each on a scale of 1-5. After 3-7 days of administration, mobility and pain improved in each animal. Amantadine was prescribed to an adult great plated lizard (Gerrhosaurus major). The dose was increased to 4 mg/kg, but results were not consistent. NSAIDs have been discontinued in one animal, and opioids discontinued in two. Currently, animals being maintained on amantadine have had no further increase in dose required, the need for additional analgesics, or further relapses of arthritic pain seen.

Key words: Amantadine, analgesia, multimodal, NMDA antagonist, osteoarthritis

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GROSS LESION RECOGNITION IN ZOO REPTILES

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Abstract

Gross lesion recognition, like other imaging modalities, is a bit of an art form. Vast zones of grey may confront the investigator when considering what the lesion is, what it could be, and what to do with it. And nobody is good at all of it: A seasoned livestock pathologist could possibly clear an entire day’s necropsies without the help of a single histology slide. A bee keeper would recognize immediately the maggot stages that parasitize his colony. A dog-cat pathologist in a busy private laboratory or a companion animal clinician may not have a clue about gross lesions in livestock or bees. Historically, attempts to accurately diagnose disease by gross lesion recognition have been a humbling experience, and that has been the impetus for all further diagnostic specialties. So now that gross pathology has been put in its proper place, why even bother with such an imperfect science? Obviously, gross lesions, or lack thereof, are the first visual indication of what may be wrong with the patient. These are the lumps, the effusions, the asymmetrical oddities, the discolorations, the odiferous clues to disease that (hopefully) stimulate a “scientific” thought process culminating in a list of differential diagnoses. The purpose of this masterclass is to present images of common and not so common reptile gross lesions in live and necropsy specimens, and in a participatory manner, establish a differential diagnosis and means for establishing a definitive diagnosis.

Key words: Diagnosis, differential, gross, lesion, pathology, recognition, reptile
REGENERATIVE MEDICINE IN LAMENESS AND ORTHOPEDICS

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Abstract

Regenerative medicine is the process of harnessing natural healing processes to improve upon tissue repair for a more functional healed tissue. The holy grail of regenerative medicine would be to recapitulate fetal development, resulting in healed tissues that cannot be distinguished from uninjured tissue. Although to date this has not been achieved in musculoskeletal tissues, the potential to substantially improve outcomes with regenerative techniques is considerable. Consequently, there has been much activity in research and widespread clinical use of regenerative therapies for equine orthopedic applications. Some of the tools for regenerative medicine in orthopedics include stem cells, platelet-rich plasma, autologous conditioned serum, growth factors, and gene therapy. Regenerative therapies can be applied by intralesional, perileisonal, intra-articular, or intravenous injections.

Key words: Advanced imaging, lameness, orthopedics, regenerative medicine, stem cell

STEM CELLS

Definition

Stem cells, unlike their somatic cell counterpart, are self-renewing, highly proliferative, and capable of multi-lineage differentiation. The ultimate stem cell is made at conception. After fertilization, the zygote consists of totipotent stem cells that are able to form all three germ layers as well as placental tissue. Once the zygote becomes a preimplantation blastocyst, the inner cell mass consists of pluripotent stem cells that will give rise to all three germ layers – ectoderm, mesoderm, and endoderm – and can no longer form placental tissues. At that stage, the stem cells are embryonic. After day 8, the cells become either somatic cells (terminally differentiated) or stem cells committed to a specific lineage (multipotent). After that point, the stem cells are considered adult derived despite their presence in fetal tissues. Local niches of lineage-committed multipotent stem cells remain in adult tissue throughout life for normal tissue remodeling and repair. With increasing age, the number, expansion potential, differentiation potential, and so-called potency of stem cells declines; therefore, there is increasing interest in allogeneic embryonic and fetal derived stem cells as well as banking of autologous stem cells from postnatal samples.

The initial enthusiasm for stem cells in regenerative medicine was one of tissue-specific differentiation, in that stem cells implanted to a cartilage lesion would engraft, become chondrocytes, and produce cartilage matrix. As both basic science and clinical data accumulates, it appears that stem cell therapy may also be, largely or in part, due to local production of bioactive molecules and immune modulation rather than tissue specific differentiation and long term engraftment. What treatment effects stem cells actually impart is an important question. The answers will likely change what conditions are treated with stem cells and by which stem cell
source, when they are applied, by which route, how often they are administered, and the dose of cells used. To answer these questions additional clinical and experimental studies are required.

Because of the difficulty in isolation, expansion, and cryopreservation of equine embryonic stem cells, they have not been investigated in the horse for regenerative medicine and will not be discussed in this chapter. In contrast, adult-derived stem cells (nonembryonic) are generally considered to be safe and to carry little risk of tumor formation, are easy to isolate and expand, and have been used extensively in the horse. Thus, this discussion will focus on adult-derived stem cells.

Adult-derived mesenchymal stem cells (MSCs) are considered an excellent stem cell source for musculoskeletal regenerative therapies because they are readily available from several tissues, allow for use of autologous cells as well as allogeneic cells because of immune tolerance to non-self MSCs, and are of mesodermal lineage and thus able to differentiate into cartilage, tendon, and bone. The immune privilege of MSCs may be in part due to lack expression of MHC class II and most of the classical cotimulatory molecules of antigen presenting cells. Recent evidence also suggests that in addition to being immune privileged, MSCs are immune-modulatory, through secretion of chemo-attractants followed by regulation of immune cell activation (T and B cells). Finally, MSCs may also be anti-inflammatory through inhibition of IFN-γ and TNF-α and stimulation of metalloproteinase inhibitors and anti-inflammatory interleukins, such as IL-10. The most exciting element of the MSC is their apparent exquisite responsiveness to their microenvironment, in that they behave according to the environment in which they are placed. In this manner, MSCs would respond appropriately to the degree of disease and modulate the local environment in favor of reduced inflammation, reduced apoptosis and/or enhanced matrix synthesis of endogenous progenitors and tissue-specific cells.

Because of their broad overlap with other cell populations, MSCs cannot yet be sorted accurately by cell surface markers. Therefore, many labs select and isolate MSCs by expanding the tissue culture plastic adherent population of colony forming cells. This translates to a culture period of 2-3 wk, in vitro, to isolate and expand MSCs from clinical samples for autogenous therapy. In the horse, MSCs have been isolated from bone marrow, adipose, tendon, muscle, umbilical cord blood and tissue, gingiva and periodontal ligament, amniotic fluid, and blood. The different tissue sources vary in the ease of harvest, expansion potential, and differentiation capacity. Several academic and commercial laboratories provide for the isolation, expansion, and cryopreservation of stem cells from several different tissue sources, namely bone marrow, fat, and umbilical cord or blood. Directions for collection and shipping procedures are available from each lab. To date, bone marrow-derived MSCs from both the horse and human have been the most thoroughly studied and have the most evidence for ability to undergo chondrogenesis, tenogenesis, osteogenesis and contribute to cartilage, tendon, and bone repair as well as modulate inflammation and soft tissue repair within the joint.

**Autologous or Allogeneic**

Autologous (self) therapy has been used most in horses to date. Use of autologous cells is considered safe, with minimal risk for disease transmission. A major disadvantage of autologous cells is that, unless cells have been banked prior to injury, their use dictates a delay of 2-3 wk for
isolation and expansion. Although many labs are offering banking of autologous MSCs, the long-term viability of cryopreserved MSCs has not been fully elucidated. One way to avoid the culture delay for autologous MSCs is to use patient-side kits to concentrate stem cells. Several commercial kits are available that enrich for the nucleated cellular portion resulting in a higher concentration of MSCs in a small volume. Another method to avoid delay would be to use allogeneic (non-self) cells.

Because MSCs are immune privileged (see above), allogeneic cells can be used in nonrelated individuals and without immune testing. Although this has been demonstrated in most species, it has not yet been thoroughly reported in the horse. Use of an allogeneic stem cell line would allow use of an ‘off the shelf’ stem cell product and would have several advantages. First, it may reduce the variability between treatments as different cultures between and amongst patients have different characteristics. Second, it may shorten the time between diagnosis and treatment. Third, it will allow for younger stem cells from fetal, adolescent, or young adult tissues to be used in aged horses, increasing stem cell potency and possibly enhancing the treatment effect. Finally, it may reduce costs by minimizing procedures, patient visits, and cell preparation time; however, allogeneic stem cells are considered a drug by the Food and Drug Administration (FDA), and as such, are required to undergo the same safety and efficacy trials and manufacturing processes that are required of pharmaceuticals. Such trials are expensive and time consuming; therefore, commercial allogeneic stem cells are not yet available. In contrast, the use of autologous stem cells in veterinary patients is not currently regulated by the FDA.

**Current Use in Orthopedics**

Stem cell use in tendon and ligament injury provides the most evidence to date for improved repair. In a report of 105 National Hunt horses with over 2 yr of followup, there was a lower recurrence rate of bowed tendon in tendons treated with stem cells (~25%), compared with traditional therapies (approximately 55%; historical controls). Other tendon injuries that are being treated are lesions of the deep digital flexor tendon in the pastern and foot and ligament injuries, including suspensory and collateral ligaments. Most often, stem cells are suspended in serum, plasma, platelet-rich plasma, bone marrow supernatant, or culture medium for direct intralesional injection under ultrasound guidance 3-6 wk following injury.

Intra-articular stem cell injection is used in horses for the treatment of acute articular injuries after surgical debridement and for the minimization of osteoarthritis (OA) progression. Several animal models of OA have shown promising results across several different research groups with reduced cartilage degeneration and OA progression and improved soft tissue healing. In the horse, there is experimental evidence for improved healing of cartilage defects treated by microfracture and anecdotal evidence for improved lameness resolution in stifle injuries, particularly those with meniscal injury, after intra-articular MSC injection. Occasional joint flare has been noted following intra-articular stem cell injection and may be related to contaminating foreign substances from the culture medium or injection of dead cells. For intra-articular injection, stem cells are suspended in plasma, serum, or bone marrow supernatant, with or without hyaluronic acid, but not with antimicrobials as the doses routinely used in joint injections can be toxic to cells.
Direct arthroscopic implantation of MSCs into joint defects when treating osteochondritis dissecans (OCD), osteochondral injury, or cyst-like lesions has been used in the horse experimentally and clinically, with improved outcomes. In this application, stem cells are implanted within a scaffold, or a three-dimensional matrix such as an autologous fibrin clot, to maintain them within the articular cartilage defect. One scenario that would not necessitate a scaffold would be injection of stem cells under an OCD flap that is being salvaged by arthroscopic pinning.

Stem cells, especially from bone marrow, have robust bone-forming potential and may prove to be an important breakthrough in equine fracture fixation and arthrodesis. Through an increased rate of bone production, stem cells may help to achieve adequate healing prior to implant loosening or fatigue failure. Application of stem cells to fractures sites is most often done in a scaffold, to maintain the cells at the site of fracture.

**PLATELET-RICH PLASMA**

Following wounding, circulating platelets accumulate and become activated when exposed to a basement membrane. Activation causes platelet degranulation and release of many bioactive substances that promote healing, stimulate angiogenesis, recruit endogenous stem cells, and regulate inflammation. Specific growth factors released from activated platelets at high concentrations include platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin like growth factor (IGF), and vascular endothelial growth factor (VEGF). Platelet-rich plasma (PRP) is a fraction of blood with an increased platelet concentration two to four times baseline, and is used largely for its anabolic properties. Other components of PRP are plasma proteins dissolved in water (adhesive proteins, clotting factors, fibrinolytic factors, proteases and antiproteases, basic proteins, and membrane glycoproteins), varying concentrations of leukocytes, and sporadic erythrocytes and stem cells.

The principal advantage of PRP is that production can be performed patient-side for immediate use, relatively inexpensively. Platelet-rich plasma is produced through centrifugation or filtration of venous blood and the procedure can generally be accomplished within 15 min. The blood collection and preparation procedure varies from manufacturer to manufacturer and will influence the composition and volume of the product (platelet and leukocyte fold change, for example). It is likely that the varying reports of efficacy in clinical outcomes are influenced by PRP composition and the ideal concentration of platelets and leukocytes within PRP remains undefined. Certainly, as platelet concentration increases, so does growth factor concentration. Therefore, higher platelet concentrations may be desirable. In support of this view, in vitro tendon explant data shows that tendon and ligament gene expression was improved with increasing platelet concentration. In the same study, increasing leukocyte concentration increased gene expression of collagen type III, the protein composition of scar tissue which is undesirable.

Extra doses of PRP can be stored frozen (-20°C) for later use. It is important to note that leukocytes within the PRP will be lysed and platelets will be activated by this storage process. While some have recommended platelet activation of fresh PRP with varying additives (calcium chloride, thrombin) or by freezing, it is probably not necessary as the local environment should be sufficient.
to activate platelets for growth factor release. If PRP is to be used as a clot, addition of thrombin and calcium is required. Clinical evidence suggests that PRP is useful for acute tendon and ligament injury when injected intra-lesionally under ultrasound guidance into acute to sub-acute lesions. Platelet-rich plasma has also been used for arthropathy and delayed bone healing. Anecdotally, joint flares have been reported following PRP injection and may be related to the platelet-to-leukocyte ratio and leukocyte concentration.

**AUTOLOGOUS CONDITIONED SERUM**

Autologous conditioned serum (ACS) therapy was developed to counteract the inflammatory mediator, interleukin-1, with a naturally occurring antagonist protein, interleukin-1 receptor antagonist protein (IL-1Ra; IRAP). Inhibition of interleukin-1 provides an analgesic as well as an anti-inflammatory effect and thus ACS is used for its anticatabolic properties. Kits are commercially available for production of ACS, in which blood is incubated overnight in the presence of medical grade glass beads. This incubation leads to the de novo synthesis and release of stored endogenous substances, including IL-1Ra, by leukocytes and platelets in the blood. Twenty-four hours later, the sample is centrifuged and the supernatant (serum) is collected, sterile filtered (0.2 µm filter), and separated into several aliquots. A portion (usually about 2 mL) of the ACS is injected into the affected region and the extra doses can be stored in a freezer (-20°C) for approximately 1 yr. Although IL-1Ra is the target protein made in this process, ACS probably contains a large and diverse set of factors that make it effective.

There has been widespread use of ACS in horses, primarily via intra-articular injection in the treatment of joint disease, osteoarthritis, or synovitis. Joint flare, or serious adverse reaction to joint injection, appears to be infrequent but has occurred. Practitioners have also used ACS for intralesional tendon or ligament injection. Anecdotal evidence suggests that the majority of horses that receive and respond to ACS are those that have become refractory to intra-articular steroids except on a very frequent reinjection schedule. Timetables employed for ACS therapy vary among practitioners. Some administer each injection weekly for 3-4 treatments, and others administer each injection monthly.

**GROWTH FACTORS AND GENE THERAPY**

Addition of growth factors, either directly as proteins or indirectly through gene therapy techniques to stimulate their production, has been used in several orthopedic applications. Compared to injection of protein, which has a very short half-life, gene therapy would allow for continued expression of the transgene, increasing the duration of growth factor exposure. Members of the transforming growth factor (TGF) family of growth factors and insulin like growth factor (IGF) have been used via proteins or gene therapy in the joint to stimulate synthesis of hyaline cartilage, improve subchondral bone architecture, and inhibit inflammatory responses; bone morphogenetic bone morphogenetic (BMP) protein and gene therapy has been used in fractures and cyst-like lesions to stimulate bone production; IGF protein and gene therapy has been used in tendon lesions to stimulate repair; growth hormone releasing hormone (GHRH) gene therapy has been used in the treatment of laminitis; and IL-1Ra gene therapy has been used in the joint to minimize inflammation.
Many methods for gene transfer are available. For satisfactory transduction efficiency and effective protein expression, the best described gene therapy procedures involve use of viral vectors such as retrovirus, adeno-associated virus, adenovirus, and many others. In the horse, use of adeno-associated virus and adenovirus has been reported. Nonviral methods are also available but have been less studied. Of the virally mediated gene therapy techniques, there is great variability in the genomic integration and subsequently the duration of transgene expression. It is unknown whether a long duration of transgene expression or permanent transgene expression would be required in orthopedic applications. Gene therapy techniques are not yet available for clinical application, but may become a routine part of practice in the future.

CONCLUSIONS

There is still much to learn about the optimal treatment paradigm in regenerative therapies including indications, technique, route, dose, timing, and frequency. There are several factors that contribute to the lack of evidence. First, most regenerative techniques are unencumbered by federal regulations and as such are being employed for a variety of conditions, by differing manufacturing processes and with differing treatment regimens. Second, autologous regenerative products have differing compositions, both between patients and even within the same patient from different collections. Such widespread use of a variable product makes it increasingly difficult to make sound conclusions. Based on the anti-inflammatory effects and the ability of regenerative techniques to orchestrate tissue repair and regeneration via endogenous cell recruitment and trophic factors, early treatment and possibly repeated treatments may be advantageous.

Stem cell therapy has successfully undergone proof of concept testing in equine tendon, cartilage and intra-articular musculoskeletal applications. After the success of initial studies, the enthusiasm for stem cells in regenerative medicine was one of tissue-specific differentiation, in that stem cells implanted to a cartilage lesion for example, would engraft, become cartilage cells, and produce cartilage matrix. As both basic science and clinical data accumulates, it appears that the effectiveness of stem cell therapy may also be, largely or in part, due to local production of growth factors and anti-inflammatory factors. What treatment effects stem cells actually impart is an important question and continued research is warranted. The answers found by continued research will likely change what conditions are treated with stem cells and by which stem cell source, when they are applied, by which route, how often they are administered, and the dose of cells used. Combined with good diagnostics, surgical care when indicated and a careful rehabilitation program, stem cell therapy is helping equine athletes with musculoskeletal injuries to return to, and stay in, the same level of performance as they were in prior to injury.
HOW, WHAT, AND WHY OF TRACKING THE ACUTE PHASE RESPONSE

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Abstract

Tracking the acute phase response has long been a goal of protein electrophoresis. The use of immunoassays to quantitate specific acute phase proteins has been well described in companion and large animals and rodents. In more recent years, commercial reagents have been studied for their reactivity to avian and exotic species and nondomestic mammals. There are clear applications for these biomarkers in health assessments and prognosis. The how, what, and why of acute phase protein testing continues to be a work in progress for many species.

Key words: Acute phase protein, acute phase response, C-reactive protein, haptoglobin, protein electrophoresis, serum amyloid A

INTRODUCTION

The acute phase response (APR) is an integral part of the innate immune system. The APR is a complex systematic inflammatory process that begins with a local stimulus such as a response to injury or infection and then the production of cytokines which signal the liver to initiate or upregulate the expression of acute phase proteins (APP). It has been estimated that more than 200 APP may be produced during the APR. APP have unique roles including opsonization, complement activation, and enhancement of phagocytosis (C-reactive protein, CRP); enhancement of chemotaxis and aid to tissue repair (serum amyloid A, SAA); and binding hemoglobin to minimize oxidative activity (haptoglobin, HP). These proteins have been found to highly conserved with observations of these or similar proteins from mollusks to turtles to mammals.

APP are classified as positive or negative. Major APP can increase 10-1000-fold. The increase is rapid rising from near negligible levels and occurs within 16-24 hr. In most companion and large animals, SAA and CRP act as major APP. Mild and moderate APP are present in detectable levels in normal animals. Increases may be 2- to 10-fold for moderate APP and less than 2-fold for minor APP. Expression is often delayed to 4-6 days after insult and elevated levels will persist after resolution of the inflammatory process. HP is often defined as a minor to moderate APP in most species.

Albumin is classified as a negative APP as it decreases in ongoing APR. In some mammals, transferrin has also been classified as a negative APP. Transferrin has been defined as a positive APP in avian species.
VALUE OF TRACKING THE ACUTE PHASE RESPONSE

APP greatly contrast the sensitivity of other traditional measures of inflammation including white blood cell count, neutrophil count, and the A/G ratio. These independent measures have been conducted in dogs and horses. In dogs with various inflammatory processes ($n = 900$), a correlation of $r = 0.44$ between WBC counts and CRP was reported. In horses, the correlation between WBC counts and SAA was weak ($r = 0.11$). In the latter study, SAA had the highest diagnostic accuracy for inflammation versus the other measures at 75%. In a large study of clinically ill cattle, animals were classified as experiencing either acute or chronic inflammation. SAA levels were found to have 100% sensitivity and HP levels showed 76% specificity. Neutrophil counts showed 30% sensitivity and 7% specificity. It should be noted that these comparisons are biased by the different timelines of expression of each measure.

When strong agents of inflammation such as turpentine oil are injected into laboratory animals, there can be a mild transient increase in WBC; however, a true leukocytosis is not observed until 4-7 days later. Serum albumin will decrease but will not reaching minimum concentrations until day 5. In contrast, CRP and SAA increase several hundred fold by 48 hr. An impressive characteristic of major APP is the rapid decrease in levels as the insulting stimuli is addressed. With a short half-life and negative feedback, the levels can drop within a few days. The rapid increase and rapid decrease provide key value to the use of APP as prognostic indicators.

In some cases, elevated APP levels can aid in a differential diagnosis. In horses with serious enteritis, colitis, or peritonitis, 50-fold higher SAA levels were observed versus horses with obstruction, perforation, or ulcers. In a large study of horses with various infections, the highest SAA levels were consistently observed with bacterial infections including bacterial pneumonia. In the aforementioned study on clinically ill cattle, acute illnesses were associated with SAA expression whereas the slower forming HP response was associated with chronic processes.

As the origin of the APP stimulus may be infection, inflammation, trauma, stress or neoplasia, APP are rarely solely diagnostic for any one disease or etiology.

MEASURING ACUTE PHASE PROTEINS

An ongoing APR can be reflected in fraction changes in protein electrophoresis. Classic changes have been observed with feline infectious peritonitis, ehrlichiosis, and myeloma in companion animals. Newer automated and semi-automated methodologies including enzyme-linked immunosorbent assay, immunoturbidity, and colorimetry has been employed to quantitate specific APP. Many of these reagents are used in assays to measure human APP and have variable cross reactivity in animals. In the antibody based protocols, methods and reagents must be individually validated for each species. If reagents are identified as cross reactive based on a sample set of clinically normal and abnormal animals, traditional studies of coefficient of variation analysis and linearity under dilution must be undertaken. From that point, the study can then include a large number of samples to determine reference intervals and assess the clinical impact of using these biomarkers with patients. In limited instances, there are species specific reagents; these often are in ELISA format.
BIRDS

Studies in chickens have dominated the literature on APP expression in birds. With injection of gold standard inflammatory agents, changes in SAA, transferrin, PIT-54 (analog of HP), and alpha-1 acid glycoprotein have all been documented. In the author’s laboratory experience, finding reagents with wide avian species cross reactivity has been difficult although the colorimetric reagents used to quantitate HP appear to be valid. Increased SAA expression has been documented in falcons with aspergillosis. It appears that this APP is a major APP in this species.

The current impression is that protein electrophoresis provides very good sensitivity for ongoing APR. It provides a broad impression of different globulin changes and specifically quantitates albumin which is an important and sensitive negative APP in birds. In a comparison of total WBC counts versus EPH abnormalities in African grey parrots (judged by a change in the A/G ratio), a marginal correlation was observed between significantly elevated WBC counts and significantly decreased A/G ratio (C. Cray, personal communication, April 2017). In 49% of the cases with a decreased A/G ratio, an elevated WBC count was found. Notably, 43% of the cases showed a normal WBC count when a significant decrease in the A/G ratio was present. Similarly, the majority of the cases showed a normal WBC count when a mild to moderate decrease in the A/G ratio was present. This data indicates a differential sensitivity between these two methods and supports the use of electrophoresis in routine clinical pathology investigations of avian species.

REPTILES

SAA expression (at the RNA level) was found to increase 1000-fold in soft-shell turtles experimentally infected with gram negative bacteria. To date, attempts to validate commercially available SAA antibodies in most reptiles has not been successful. Hemoglobin binding activity, quantitated by the colorimetric assay for HP, has been demonstrated in box turtles, loggerhead turtles, inland bearded dragons, and rattlesnakes (C. Cray, personal communication, April 2017). As additional reagents for major APP become available, studies should consider the relative sensitivity of protein electrophoresis vs. APP assays in these species.

SMALL EXOTIC MAMMALS

Rats and mice have interesting differences in APP expression versus companion animals; to date, analysis is best performed using ELISA methods. There has been little to no description of APP expression in guinea pigs, hamsters, and gerbils.

CRP expression has been described in rabbits with suspected Encephalitozoon cuniculi infection. Elevated CRP levels acted as an adjunct diagnostic test to improve the specificity of serologic titers against this organism and also served as a prognostic indicator. Recent studies indicate also the utility of newly described VET-SAA reagents (C. Cray, personal communication, April 2017).

Ferret SAA was cloned and found to be similar to other mammalian SAA. In studies from the author’s laboratory, SAA was found to be increased 2-5 fold in clinically abnormal ferrets. A 10-fold increase in HP was observed in a ferret with myeloma.
FISH AND SHARKS

APP expression has been well documented at the RNA level in several species of fish. Attempts to document expression in a trauma model in koi were not successful and may have been related to lack of reagent cross reactivity and experimental design. CRP has been validated for use in bonnethead sharks. A 2.5-fold mean increase was found in clinically abnormal sharks which included cases of bacterial septicemia and suspected Fusarium infection. CRP also has been validated in sand tiger and sandbar shark species (C. Cray, personal communication, April 2017).

MARINE MAMMALS

SAA expression and use in health assessments was first described in the manatee by Harr et al. The use of an automated SAA assay was described in 2013. Approximately 30-fold higher mean SAA levels were quantitated in manatees with cold stress or trauma. The sensitivity was 93% and specificity was 98% which were superior to total white blood count and albumin quantitation. Additional studies have been completed in elephant seals and dolphins. The latter utilizes a dolphin specific reagent.

PRIMATES

APP expression has been documented in many species of nonhuman primates. SAA, CRP, and HP testing was validated in rhesus macaques and more than a 200-fold increase in CRP was observed in animals with chronic active inflammation associated with Mycobacterium infection. SAA was also described to be increased in macaques with hepatic amyloidosis. SAA and CRP have been found to be increased in the orangutan with various infectious and inflammatory diseases (C. Cray, personal communication, April 2017).

OTHER NONDOMESTIC MAMMALS

APP expression has been well documented in the cheetah. SAA tests have been validated and significant increases were reported in clinically abnormal animals. SAA testing has also been validated for use in lions, tigers, and leopards (C. Cray, personal communication, April 2017).

SAA and HP testing has been validated in elephants. Significant increases in SAA were observed in elephants with > 10,000 virus genome copies/ml EEHV-1 in blood. This biomarker appeared to have excellent prognostic value in elephants with this infectious disease. In addition, increases in SAA were observed in Asian elephants with pododermatitis and traumatic injuries.

There are considerable other publications in a wide variety of species including alpaca, antelope, capybara, and water buffalo. Caution should be used when submitting for APP testing as the published and nonpublished/anecdotal reports will widely vary with reagent cross reactivity. Refer to the website of the University of Miami Acute Phase Protein Laboratory (www.cpl.med.miami.edu) for a full list of lab validated species and contact the lab if you have interest in submitting samples for other species as basic testing may already be completed.
CONCLUDING REMARKS

The field of proteomics is rapidly growing so it is expected many more biomarkers will be identified to gauge the acute phase response and assist in the diagnosis of specific diseases. In a spectroscopy study of samples from falcons with aspergillosis, 3-hydroxybutyrate is significantly altered versus normal falcons. Lipoprotein fractions were also found to be significantly altered in clinically abnormal cownose rays. These observations underlie the opportunities for further study in these areas in other species.

Reagent availability has increased in recent years making it possible to test possible cross reactivity in relatively easy fashion. Still, APP expression in some species has defied further elucidation and may necessitate a basic science approach. Of note, point-of-care options for testing are, and will continue to become, more commonplace. While the applications are especially exciting, these reagents, like those used at the reference laboratory, need to be validated species by species.

LITERATURE CITED


MODIFIED CHOUKROUN’S PLATELET-RICH FIBRIN USED AS A TOPICAL WOUND TREATMENT IN A CAPTIVE ELONGATED TORTOISE (*Indotestudo elongata*)

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Abstract

Few studies have been reported about the benefit of platelet-rich fibrin (PRF) in the treatment of reptiles.¹,² Most protocols require the use of specific anticoagulants and several steps to prepare PRF for use, but the preparation of Choukroun’s PRF is relatively easy.¹,² Choukroun’s PRF is derived by immediate centrifugation from collected blood without any anticoagulant added, which makes a strong fibrin matrix clot.¹,² In this study, the Choukroun’s PRF technique was modified to produce PRF for application on an elongated tortoise (*Indotestudo elongata*). The Choukroun’s PRF was applied once per week or every other week with a satisfactory effect. To the author’s knowledge following an electronic search of current literature, there are currently no reports of Choukroun’s PRF preparations derived from reptilian blood.³ Further studies and detailed trials are needed to evaluate the true efficacy of this protocol in reptiles. The aim of this communication is to share the clinical findings and encourage more veterinarians to institute this treatment and report their results.

**Key words:** Choukroun’s PRF, *elongated tortoise*, platelet-rich fibrin, platelet-rich plasma, reptile, wound management

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LITERATURE CITED


ATLAS OF INVERTEBRATE FEED SPECIES: DISTINGUISHING FRIEND FROM FOE

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Abstract

Parasites are a common finding in zoological collections. It can be difficult to distinguish between typical food species and parasites in captive animals because amphibians, reptiles, birds, and fish are fed various species of worms and insects (e.g., earthworms, fruit flies, crickets). The objective of this study is to create an atlas by which normal food species and parasites can be distinguished. Commonly used invertebrate food items from the diets of captive reptilian, amphibian, avian, and aquatic species were collected from three zoological facilities, fixed in 10% formalin, and processed routinely for histology. Microscopic sections of common invertebrate food items were examined to identify specific characteristics of each organism. Organization of musculature, cuticle composition, and appendages are among the features that can be used to distinguish between parasites and common invertebrate food species. For example, earthworms have two layers of musculature (circular and longitudinal), which distinguish them from many parasitic nematode species. The features identified during this study can be used to evaluate the gastrointestinal contents of captive and wild reptiles, amphibians, fish, and birds submitted for necropsy.

Key words: Food, histology, invertebrate, parasite

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LITERATURE CITED


EVALUATION OF THE USE OF KETAMINE-DIAZEPAM COMBINATIONS FOR IMMOBILIZATION OF AFRICAN LAND TORTOISE (Geochelone spp.)

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Abstract

Zoo and wildlife veterinarians and animal caregivers are constantly being exposed to persistent dangers when wild animals are restrained. This can result in physical attacks and possible spread of zoonoses in humans and in wild animals. Various forms of injuries and stress have been reported. Chemical immobilization in reptiles is unpredictable because of the ectothermic nature of the animals and this has been said to be the cause of prolonged recovery from anesthesia. A safe and effective anesthetic protocol is essential for proper immobilization of chelonians and other reptiles. The anesthetic effects of concurrent administration of varied doses of ketamine with varied doses of diazepam were evaluated in 16 healthy land tortoises (Geochelone spp.). The animals were divided equally into four groups labelled A-D. Each group was administered a combination of ketamine and diazepam intramuscularly as follows: animals in group A received a combination of 44 mg/kg ketamine with 0.25 mg/kg of diazepam, animals in group B received 22 mg/kg ketamine with 0.25 mg/kg of diazepam, animals in group C received 44 mg/kg ketamine with 0.5 mg/kg diazepam, and animals in group D received 22 mg/kg ketamine with 0.5 mg/kg of diazepam. Anesthetic effects were monitored every 10 min by using a pair of forceps to determine the animal’s ability to retract limbs when extended. Full extension of head and limbs was achieved within mean periods of 10 min, 15.5 min and 13 min for groups A, C and D, respectively. Animals in group B had only partial retraction and did not reach full extension of head and limbs; they were only sedated. Full recovery occurred in mean periods of 118 min, 25 min, 108 min and 132 min for groups A, B, C, and D, respectively. For surgery that may last more than 1 hr, the combinations administered for groups A, C, or D may be used; whereas, the combination administered in group B could only be used for sedation.

Key words: Anesthesia, diazepam, Geochelone spp., ketamine

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LITERATURE CITED


TO STAB OR TO SHOWER? ALFAXALONE ANESTHESIA IN COLORADO RIVER TOADS (Incilius alvarius) BY INTRAMUSCULAR INJECTION OR TOPICAL APPLICATION

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Abstract

Veterinary diagnostics often require the immobilization of amphibians; however, intramuscular administration of anesthetics may be harmful to amphibians given their thin epidermis. Alternatively, topical application or immersion may be less effective in desert-adapted species. Seven adult Colorado River toads (Incilius alvarius) were administered 20 mg/kg of alfaxalone either intramuscularly (i.m., n = 4) or by topical application (t.a., n = 3). All animals belonged to a chronically Mycobacterium spp. infected population and were to be culled following anesthesia achieved in this trial. Time to recumbency, heart and respiratory rate, response to palpebral, corneal, nociception (toe pinch) and righting reflexes were evaluated and repeated every 5 min following induction. Anesthetic depth on a scale of 1 to 5 (no effect to excessively deep anesthesia) was recorded every 5 min. If no major effect was noted following 30 min, alfaxalone was repeated at 20 mg/kg. Recumbency was achieved only in the i.m. group (median 12 min, range 7-14 min). No loss of corneal, palpebral, nociception (toe pinch) and righting reflexes were achieved in the t.a. group despite repeated alfaxalone administration; whereas, these were absent only to a variable extend in the i.m. group. Anesthetic depth and heart rate differed significantly between groups throughout anesthesia as well as at time points of attempted blood sampling (P = 0.03). Undesired side effects were skin irritation (n = 1) in the t.a. group and intermittent apnea (n = 1) in the i.m. group. Intramuscular alfaxalone at 20 mg/kg may therefore be suitable for short noninvasive procedures whereas topical application cannot be advocated and alternative routes are recommended.

Key words: Alfaxalone, anesthesia, Colorado River toad, Incilius alvarius, intramuscular injection, topical application
PHARMACOKINETICS OF SUBCUTANEOUS HYDROMORPHONE ADMINISTRATION IN BEARDED DRAGONS (Pogona vitticeps)

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Abstract

Opioids are commonly used for pain management in reptiles, though few pharmacokinetic studies exist to determine effective dosages. Hydromorphone antinociceptive efficacy was previously demonstrated in red-eared slider turtles using a thermal noxious stimulus device.1 The objective of this study was to determine the pharmacokinetics of two subcutaneously administered dosages of hydromorphone (0.5 and 1.0 mg/kg) in six bearded dragons (Pogona vitticeps). The maximum concentration (Cmax) for subcutaneous administration at 0.5 and 1.0 mg/kg was 211 and 556 ng/ml, respectively and both peaks were detected 30 min post hydromorphone administration. Hydromorphone administered subcutaneously at both dosages provided measurable plasma concentrations of hydromorphone for up to 12 hr with the longest measurable concentration detected at 24 hr. Clinical signs of sedation were observed for up to 1 hr post hydromorphone administration for most bearded dragons (5/6) when hydromorphone was administered at 1.0 mg/kg. Plasma concentrations of hydromorphone were detectable for up to 24 hr post administration in 5/6 bearded dragons receiving 1.0 mg/kg.

Key words: Analgesia, bearded dragon, hydromorphone, opioid

LITERATURE CITED

ARE CROSS-SECTIONAL HEALTH DATA REALLY GIVING US ENOUGH INFORMATION TO CHARACTERIZE POPULATIONS? ENHANCING CHELONIAN HEALTH THROUGH A PROSPECTIVE COHORT STUDY

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Abstract

Reptile populations worldwide are at risk from multiple stressors, including habitat loss and degradation, invasive species, unsustainable use, and emerging infectious diseases.\(^1\) Chelonian health is evaluated by parameters such as hematology, pathogen presence, and clinical pathology; however, few studies exist observing these parameters within individuals over time. To address this deficit, Eastern box turtles (Terrapene carolina carolina, \(n = 22\)) were affixed with radio telemetry units at a site with recurrent ranavirus mortalities. Blood samples were collected every other week from May through November 2016 and evaluated for hematologic parameters. Total white blood cell counts varied throughout the year and were observed to be higher in females, but no differences were observed based on season or average daily movement. Directly observed turtle movement did not appear to be affected by any hematologic factors, but mainly driven by environmental factors, specifically warmer temperatures and rain. Individual turtles tested positive for Terrapene herpesvirus 1, box turtle Mycoplasma sp., and adenovirus, but no individual factors were significant predictors for pathogen detection. Conversely, pathogen presence was not a significant predictor for turtle health parameters when treating each turtle as nonindependent with repeated measures. Data collection over another active season should enhance ability to elucidate potential relationships among these parameters. The approach to integrate ecological and natural history factors of populations with patterns of individual health is critical to better assess population health. Furthermore, this approach can be adapted for other free-ranging and captive populations to understand temporal variation in health.

Key words: Eastern box turtle, health, hematology, movement, prospective cohort, Terrapene carolina carolina

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LITERATURE CITED

COMPARISON OF ALFAXALONE, TILETAMINE/ZOLAZAPAM, AND DEXMEDETOMIDINE INJECTABLE PRE-ANESTHETICS AND IMMERSION IN TRICAINES METHANESULFONATE (MS-222) FOR SURGICAL LEVEL ANESTHESIA IN THE HOUSTON TOAD (*Anaxyrus houstonensis*)

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Abstract

Thirteen clinically healthy Houston toads (*Anaxyrus houstonensis*), scheduled for euthanasia due to exposure to *Chlamydia* or *Mycobacterium*, were divided into three groups and used in a 3-wk cross-over anesthesia study comparing three injectable pre-anesthetic protocols (alfaxalone 15 mg/kg i.m., tiletamine/zolazepam 5 mg/kg i.m., and dexmedetomidine 0.1 mg/kg i.m.) followed by immersion into tricaine methanesulfonate (MS-222) to achieve anesthesia. Heart rates and respiration rates were recorded before injection and every 5 min post injection. Ten minutes after injection, toads were tested for righting reflexes and graded 1-3 (1-no righting response, 3-full righting response). Toads were then partially immersed into a buffered MS-222 solution at 800 ppm for 10 min. Ten minutes after immersion in buffered MS-222, toads were tested for righting reflexes and if none were noted, a toe pinch was used to evaluate deep pain sensation. Toads were graded 1-3 (1-no righting response or deep pain sensation, 3-full righting response). No complications were observed and all toads recovered within 24 hr. Tiletamine/zolazepam showed the least change in heart rate over the 20-min trial; whereas, dexmedetomidine showed a profound decrease in heart rate. Respiration rates were similar for all three groups. Dexmedetomidine produced minimal sedation. Both alfaxalone and tiletamine/zolazepam resulted in sedation, but not at a surgical level, even after a 10-min immersion into MS-222.

Key words: Alfaxalone, *Anaxyrus houstonensis*, anesthesia, dexmedetomidine, tiletamine/zolazepam, tricaine methanesulfonate
LIMITATIONS AND IMPACTS OF THE RENAL PORTAL SYSTEM ON THE DETERMINATION OF THE GLOMERULAR FILTRATION RATE USING CONTRAST-ENHANCED COMPUTED TOMOGRAPHY AND PLASMA CLEARANCE OF IOHEXOL IN BEARDED DRAGONS (*Po gona vitticeps*)

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Abstract

Due to unique anatomy, physiology, and interspecific variation, renal disease can be challenging to diagnose and treat in reptiles. The glomerular filtration rate (GFR) is an indicator of renal function and has been commonly used in human and veterinary medicine. The plasma clearance of iohexol (PCI) and contrast-enhanced computed tomography (CT) have been used to determine the GFR in healthy and diseased individuals.1-7 This pilot study was initially designed to determine the GFR of bearded dragons (*Pogona vitticeps*) using contrast-enhanced CT and PCI. Ten bearded dragons were used for this study. Dynamic images of the kidneys were obtained sequentially every 2.5 sec for 2 min after an intravenous injection of iohexol (600 mg/kg) via a catheter placed in the ventral coccygeal vein. Time-attenuation curves were constructed and GFR was obtained using Patlak plot analysis. Individual kidney and global GFR were calculated. Plasma iohexol concentration was measured at three time points (4 hr, 8 hr, and 12 hr after iohexol administration) using high-performance liquid chromatography and GFR were calculated. The average global GFR was 1.52 ± 0.98 ml/min/kg using contrast-enhanced CT and 0.24 ± 0.06 ml/min/kg using PCI. The correlation between the two methods was poor. The renal portal system, and at times the lymphatic system, could be visualized on CT images and evidently impacted GFR calculations using both techniques. This study highlights the limitations for GFR measurements in bearded dragons, and raises questions on the reliability of the ventral coccygeal vein for research purposes such as pharmacokinetic studies.

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Key words: Bearded dragon, computed tomography, glomerular filtration rate, kidney, *Pogona vitticeps*, renal portal system

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LITERATURE CITED


CYCLOBENZAPRINE AS A POTENTIAL MEANS OF PHARMACOLOGIC MANAGEMENT OF STEREOTYPIC BEHAVIORS IN BEARS

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Abstract

Stereotypic behaviors or repetitive, invariant behaviors with no obvious goal or function, are a concern for the management of captive bears.1-5 Fluoxetine is the only drug with published data on treating bear stereotypies.6,9 Recent human studies suggest that cyclobenzaprine may prevent recurrent thinking, a common component of post-traumatic stress disorder (PTSD).7 Stereotypic behavior in animals can be triggered by stimuli similar to PTSD in humans.7,8 It is thought that improved sleep allows natural coping mechanisms to decrease the repetitive thought processes associated with PTSD and stereotypic behaviors.7,8 The subject of this pilot study was a 19-yr-old male intact American black bear (Ursus americanus) that showed extensive stereotypic behaviors including pacing, swaying, chomping, head bobbing, restlessness, decreased foraging, and atypical, limited sleep. Continuous video surveillance was utilized to evaluate the incidence of behaviors. Prior to therapy, the bear displayed stereotypic behaviors during 44% of daylight hours. Cyclobenzaprine was administered (0.12 mg/kg [30 mg]) orally once daily with food at 7:00 pm. Stereotypic behaviors decreased by 70% with therapy. Foraging behaviors occurred only 2% during daylight hours prior to therapy, but increased by 767% with therapy. The bear interacted with enrichment items, frequently slept 5-6 hr without pacing at night, and was observed taking daytime naps with cyclobenzaprine therapy. These normal behaviors were not observed prior to therapy. Given recent evidence that cyclobenzaprine can alleviate signs of PTSD in humans, this study demonstrated that cyclobenzaprine reduced stereotypic behavior in a black bear and substantially increased natural behaviors, including sleep and foraging.

Key words: American black bear, cyclobenzaprine, stereotypic behavior, Ursus americanus

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Abstract

Beginning January 1, 2016, all animals were transferred into Disney’s Animal Kingdom using a new risk-based process. This decision was based on recent publications suggesting the need for updated professional standards for preshipment testing and quarantine protocols due to the evolution of zoological health practices.1,2 The new risk-based process relies on identification of pathogen hazards and assessing and mitigating the risk to the receiving institution. If sufficient mitigation occurs prior to shipment, then mitigation after arrival (quarantine isolation period and testing) can be eliminated. This elimination removes a previously highly monitored transition period (quarantine keeper observation and care). In order to ensure that mortality did not increase with the elimination of this transition period, while still protecting the collection from disease introduction, time to and cause of death following acquisition were identified and compared for birds transferred in pre and post risk-based process use for the period 2013-2016. Percent mortality of birds deceased within 6 mo of acquisition was compared pre and post risk-based process use. Statistical analysis revealed the probability of mortality is not significantly different (P = 0.39) between standard quarantine protocols with a highly monitored transition period and a risk-based approach with entry directly into the collection. In addition, no transmissible pathogens of concern were introduced with acquired birds using either method. The implementation of a risk-based approach to animal transfers between zoos and aquariums may be an alternative method that does not pose a greater risk to animal health while also protecting the collection from disease introduction.

Key words: Animal transfer, avian, preshipment testing, quarantine, risk analysis, zoos

ACKNOWLEDGMENTS

Authors would like to thank the cast of Disney’s Animal Kingdom for their support of this project with special thanks to the Animal Health, Animal Care, and Science Teams with special thanks to Susan Feltman, Lynn McDuffie, and Dr. Mandi Schook for their assistance with data collection, organization, and analysis.

LITERATURE CITED


INCIDENCE OF CLAW DISORDERS IN GERIATRIC CAPTIVE SLOTH BEARS (Melursus ursinus) AND ITS MANAGEMENT

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Abstract

The present study describes the occurrence and management of claw disorders in geriatric (≥ 16-yr-old) captive sloth bears (Melursus ursinus) at Agra Bear Rescue Facility, Wildlife SOS, Agra, UP, India. Sloth bears are insectivores found mostly in the southern part of India, and have an average lifespan of 25 yr in captivity. Members of the family Ursidae have five digits on each limb, and each digit has a claw. Sloth bears have long, curved front claws that are approximately 7 cm in length and help them break open termite mounds and climb trees. Hind claws are shorter, measuring about 3 cm in length.2,3

Eight aged sloth bears at the Agra Bear Rescue with claw deformities and recurrent secondary traumatic wounds were selected for the study. Histories of the selected bears were collected, and field observations were performed for 1 yr. Common clinical signs included recurrent inflammation, irregular keratinization, excessive claw curvature, and ventral cavitations. Affected geriatric bears showed limited field accessibility and reduced instinctive behaviors, like scratching and digging holes, and half (n = 4/8) had abnormal gait with arthritis. Four of the bears had secondary traumatic wounds of the fore-claws. Claw wound healing took 20-30 days for complete granulation and epithelization, but new nail growth was rarely seen. Culture of wounds identified Salmonella sp., Shigella sp., and E. coli as secondary bacterial contaminants. Success of preventing secondary traumatic claw wounds was dependent on management practices followed. Onychetomy (declawing)4 was mandatory to prevent pain and retrograde infection in some cases. Preventive management practices such as periodic claw trimming by positive reinforcement, topical fly repellents in cavitated claws, provision of suitable muddy substrate to avoid paw abrasions and special enrichments to promote wear and tear of claws have been implemented.1

Claw deformities were seen in bears more than 16 yr of age and proved to be an age-related phenomenon. Severity of the disorder was specific to health of the individual bear. In conclusion, claw disorders and deformity were good indicators of age in captive sloth bears, and periodic claw trimming was the key management practice to improve geriatric animal care.

Key words: Bear, claw disorders, geriatric, Melursus ursinus, onychetomy

LITERATURE CITED


CAPTIVE BEAR WELFARE

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Abstract

Captive bears may exhibit behavioral abnormalities. While some appear to adapt to captive situations, others may develop obvious behavioral issues or coping mechanisms. Studies indicate species of carnivore with the largest ranges and long travel distances are most likely to exhibit stereotypies in captivity. Bears generally have large ranges in their natural habitats and travel extensively to obtain food. Medical or dental problems, reproductive frustration, or boredom from a lack of stimulation may also lead to the development of stereotypic behaviors. Bears with stereotypies should receive physical examinations and appropriate veterinary medical work-ups to rule out chronic or painful conditions. A behavioral assessment also is essential.

Key words: Bear, Tremarctos ornatus, regulations, Ursidae, Ursus maritimus, welfare

ANIMAL WELFARE REGULATIONS FOR BEARS

All species of bears, except polar bears (Ursus maritimus), are covered in the Animal Welfare Regulations listed under Subpart F. Regulations specific to polar bears are included in Subpart E, marine mammals. Subpart F covers the humane handling, care, treatment, and transportation of warm-blooded animals other than dogs, cats, rabbits, hamsters, guinea pigs, nonhuman primates, and marine mammals. Housing regulations including ambient temperature, ventilation, lighting, drainage, shelter from sunlight and inclement weather, and space are addressed in Subpart F of the Animal Welfare Act Standards. Feeding, watering, sanitation, employees, separation, and all aspects of transportation are also covered in Subpart F. Regulations specific to polar bears are found in the Animal Welfare Regulations Subpart E, §3.104 (e). They state primary enclosures must have a pool of water, a dry resting and social interaction area, and a den. There must be enough shade to accommodate all polar bears housed in the primary enclosure at the same time. The den must be positioned so the viewing public will not be visible to the bears from the interior of the den.

Space

The Animal Welfare Act regulations require sufficient space for each animal to make normal postural and social adjustments with adequate freedom of movement. Since this is a performance-based standard, a determination of compliance with this requirement is made by U.S. Department of Agriculture inspectors at the time of inspection, with the animal(s) present.

Providing adequate space for bears not only gives them more incentive to exercise, but may help to prevent stereotypies from developing. Species of bears that maintain large territories in the wild, such as polar bears, as well as bears that naturally travel large distances daily to forage are
more likely to develop pacing behaviors or other stereotypies. Vertical space is important for many species of bears, especially arboreal species such as the spectacled bears (*Tremarctos ornatus*), and climbing structures offer opportunities for the bears to perform all of their natural behaviors and may improve their daily activity levels. Bears may also be encouraged to explore and increase their daily activity when food incentives or other positive reinforcements are introduced into their environment. Training animals to move from one area to another promotes exercise and may help to prevent or extinguish repetitive behaviors. While polar bears must be provided large pools in which to swim, all bears benefit from having pools in their enclosures, and swimming should be considered one of their natural behaviors.

**Substrate**

Substrate composition is an important consideration for bears. Housing them on natural substrates such as grass, dirt, or sand, or using wood or supple engineered floors is preferable to concrete. Strategically placing substrates that yield to weight in areas where bears spend time or pace may help to prevent arthritis or other injuries.

**Feeding**

Many species of bears are omnivores and have nutritional requirements similar to domestic dogs. Some species of bears have specialized diets. Natural diets for most bears include a wide range of items including fish and insects to a variety of plant material. High-quality dog kibble may provide adequate nutrition when fed with other appropriate food items, and could be scattered in enclosures as enrichment and to promote foraging behaviors. Commercial complete raw meat diets designed for carnivores also may be used to satisfy nutritional needs of bears. Previously frozen fish must be supplemented with vitamin E and thiamine. A recommended diet for bears includes about 50% balanced commercial product and 50% vegetables, greens, fruits, seeds, nuts, insects, meat and/or fish. Diets must be adjusted in response to seasonal changes in physiology and allow bears to feed to satiety during fall hyperphagia. Bears are efficient at storing energy and feeding diets with higher fat content such as kibble, meat, or fish may lead to obesity. Body weight and body condition should be monitored regularly. Fruit, grain, or all-meat diets (with no bones) that are not supplemented with calcium and appropriate vitamins will lead to crippling nutritional diseases. A calcium: phosphorus ratio imbalance may cause growth problems or metabolic bone disease. Bears maintained on optimal diets and provided with appropriate supplements will have fewer health and welfare issues. Obese bears may suffer with premature joint problems or other obesity-related medical problems.

**Enrichment**

In addition to essential enclosure furnishings that allow for normal social and postural adjustments, such as digging, denning, swimming and climbing, provision of enrichment items is a best practice. Enrichment is not mentioned in Subparts E or F of the USDA Regulations and Standards; however, institutions housing bears are encouraged to provide thoughtful enrichment to the bears. Enrichment may include species-specific elements in the enclosures such as toys, food puzzles, rotating new scents within the environment, and logs. Toys should be carefully chosen and indestructible. Plastic drums that float or are partially filled with water have been successful
enrichment for polar bears. Sturdy plastic balls and plastic puzzle feeders designed for bears tend to make safe toys. All toys but should be checked regularly to ensure integrity. Other enrichment may involve placing food frozen into blocks into the exhibit. One way to enhance the effect is by periodic rotation of enrichment items. Compatible cage mates also offer enrichment; however, incompatible pairings may result in stressful situations, fights, and even mortality. Training bears to perform simple behaviors may also be considered enrichment because it involves something different for the animals to do each day.

LITERATURE CITED


FREEDOMS, PROVISIONS, AND AIMS: IMPROVING CONCEPTUAL FRAMEWORKS FOR ZOO-ANIMAL WELFARE

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Abstract

The Five Freedoms have been widely used as a guiding framework for animal welfare in many species, including zoo animals. This framework presents a list of broad ideals, rather than specific requirements with regard to the physical and emotional wellbeing of animals. Furthermore, despite being phrased as categorical statements, it is generally understood that in most cases, these freedoms can only be relatively fulfilled. As such, the Five Freedoms often fail to provide concrete guidance regarding the definition and assessment of welfare. Mellor (2016) has approached this shortcoming by proposing that the Five Freedoms be re-conceptualized as provisions and aims within the broader concept of quality of life, which results in more effective definitions and assessment of welfare in practice. The proposal presented in this paper constitutes an adaptation of Mellor’s framework, specifically for the assessment of zoo animal welfare. The adapted proposal identifies special considerations for zoo animals, including: methodologic constraints on collecting data to support evidence-based welfare decisions, individual and species-specific behavioral variation, a focus on choice and control as strategies to achieve positive affective states, and adequately assessing the impact of human-animal interactions on overall welfare outcomes. Two hypothetical case scenarios are discussed in order to illustrate the applicability of the framework. A potential goal of the adapted framework is to assist institutional animal welfare committees in establishing welfare priorities for animals in their care.

Key words: Animal welfare, animal welfare assessment, Five Freedoms, quality of life

LITERATURE CITED


BIOMEDICAL EVALUATION OF SAHAMALAZA SPORTIVE LEMURS (Lepilemur sahamalaza) IN THE NATIONAL PARK OF SAHAMALAZA-ILES RADAMA, MADAGASCAR

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Abstract

The Sahamalaza sportive lemur (Lepilemur sahamalaza) is a critically endangered small, nocturnal primate found only within a 10-km² area in the Sahamalaza Peninsula in northwest Madagascar. This study is the first biomedical evaluation performed on lemurs from the genus Lepilemur. Complete medical evaluations were performed on 26 wild Sahamalaza sportive lemurs (11 males and 15 females) over a period of 17 mo, to establish reference ranges for physiologic and biologic parameters. Twelve of these (five males and seven females) were examined and sampled twice during this period. The lemurs were anesthetized via remote injection (blow dart) with tiletamine-zolazepam. Each animal received a complete physical examination, including measurement of body weight, crown to rump length and long bone lengths, body condition assessment, heart rate, respiratory rate and rectal temperature. In addition, blood samples were collected for complete blood count, serum biochemical profile, 25-hydroxy-vitamin D₃, and when sample size allowed, Toxoplasma gondii serology. Fecal samples also were collected and analyzed. Three of the lemurs examined were juveniles (two males and one female) but the rest were adults. Six females were found to be pregnant on examination, but four of these also were examined and sampled when they were not pregnant. Body weights ranged from 460-1000 g. Body condition scores ranged from 2/9 (very under-conditioned) to 5/9 (ideal condition). No ectoparasites were found but 25 of 32 fecal samples collected were positive for oxyurid ova. Of the 20 serum samples that were tested for Toxoplasma gondii antibodies, only two were positive.

*Zoletil® 100, Virbac, 06516 Carros, France

Key words: Biomedical evaluation, Lepilemur, Lepilemur sahamalaza, oxyurid, Sahamalaza sportive lemur, Toxoplasma gondii

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The authors would like to thank The Wild Animal Health Fund for providing an AAZV Research Grant, and AEECL (The Lemur Conservation Association) for their generous contribution, as without these this project would not have been possible. They also thank Abaxis UK for their generous donation of i-STAT® cartridges. The authors would also like to thank Isabella Mandl for the opportunity to examine and collect biologic samples from the wild Sahamalaza sportive lemurs that she was studying. They also thank Alan Toyne, Joe Norman, Andrew Double, Zoe Greenhill, Lynsey Bugg, Will Raffety, Guy Randriatahina and all the staff past and present at the AEECL Ankarafa Research Camp for their invaluable assistance with this project.
RETROSPECTIVE HISTOPATHOLOGIC FINDINGS IN FREE-RANGING CALIFORNIA HUMMINGBIRDS

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Abstract

A retrospective histopathologic study of free-ranging hummingbird mortality in California was performed to identify mortality trends. Tissues from 57 wild hummingbirds representing five different native California species collected by the San Diego Zoo between the years of 1996-2016 or the Lindsay Wildlife Experience in 2015 and 2016 were histologically examined. Birds were either found dead or moribund at the time of submission or were euthanized due to unresolvable health issues. Long-term rehabilitated birds were not included in this study. Identified lesions were categorized by organ system and sex. The most commonly affected organ systems were the lung (67.3%), followed by the ingluvies (64.6%), and the liver (56.1%). While some birds had minimal or nonspecific lesions, a large proportion had lesions primarily attributable to trauma (22.8%); 14% had lesions associated with bacteria, fungus, or viruses; 12.3% had parasitic lesions; and 12.3% had multifactorial concurrent processes. Lesions of infectious disease included those associated with avian poxvirus, intestinal adenovirus, disseminated aspergillosis, bacterial septicemia, malaria (Haemoproteus spp.), and mycobacteriosis. The most commonly identified parasitic infestation was intestinal cestodiasis, for which there did not appear to be any significant associated intestinal pathology, though the large size of these cestodes may have impacted digestion. There was no apparent sex predilection for any of the identified lesions or processes.

Key words: California, hummingbirds, infectious, mortality, trauma

ACKNOWLEDGMENTS

The authors would like to thank the Wildlife Disease Laboratories at the San Diego Zoo and the Lindsay Wildlife Experience for providing hummingbird necropsy cases for review.
EVALUATION OF A SEMI-QUANTITATIVE LATERAL FLOW DEVICE FOR SERUM AMYLOID A IN HEALTHY AND OTOSTRONGYLUS-INFECTED JUVENILE NORTHERN ELEPHANT SEALS (Mirounga angustirostris)

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Abstract

Acute phase proteins have been demonstrated to be valuable tools in the detection of underlying inflammatory processes in numerous species. Previously, the diagnostic utility of serum amyloid A (SAA) has been shown in stranded juvenile northern elephant seals (Mirounga angustirostris), which was measured using an immunoturbidimetric (IT) assay (SAA-1) that is best implemented at the reference laboratory. The objective of the current study was to examine the diagnostic use of a semi-quantitative commercial lateral flow device (LFD) to the IT assay. Twenty-five samples from healthy animals with a median of 12.1 mg/L SAA (95% CI 3.4-14.4, range 0.1-45.0) all tested within normal limits by LFD (e.g., three lines). Twenty-two samples from animals with clinical signs of Otostrongylus infection and a median IT SAA of 328.0 mg/L reflected either moderate (two lines, n = 4) or high (one line, n = 18) levels by the LFD. Three samples representing low, moderate, and high SAA levels were subject to repeated measures (n = 6) and the results were in agreement by visual assessment. Similar to the SAA-1 reagent, the antibody used in the LFD appears to cross-react well with SAA from the northern elephant seal. Thus, the LFD may provide an opportunity for quick patient side assessment for inflammation. Initial assessment using the LFD device and submission to a reference laboratory for additional quantification using the IT SAA assay is recommended.

SAA-1, Eiken, Tokyo, Japan
LFD, OmniChek, Accuplex, Kildare, Ireland

Key words: Acute phase proteins, biomarker, lateral flow device, Mirounga angustirostris, northern elephant seal, Otostrongylus, serum amyloid A

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The authors thank the staff and volunteers at the TMMC for collecting and organizing these samples. Authors also thank the laboratory staff at the University of Miami for technical support. All events were performed under the Stranding Agreement between NOAA’s National Marine Fisheries Service (NMFS) West Coast Region (WCR) and the Marine Mammal Center (TMMC).
LITERATURE CITED


EFFECTS OF INTRAMUSCULAR ALFAXALONE IN PERMIT (*Trachinotus falcatus*)
AND SCHOOLMASTER SNAPPER (*Lutjanus apodus*)

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**Abstract**

Alfaxalone (Alfaxan®) is a synthetic neuroactive steroid, progesterone analog, which binds receptors of the inhibitory neurotransmitter gamma-aminobutyric acid in the central nervous system to induce muscle relaxation, sedation and anesthesia. The drug is FDA-approved for intravenous use in dogs and cats and has been studied in a variety of species including birds, reptiles, amphibians, invertebrates, and freshwater fish.1-7 The effects of alfaxalone administered intramuscularly in two marine fish species, permits (*Trachinotus falcatus*) and schoolmaster snappers (*Lutjanus apodus*), were studied to determine if intramuscular (i.m.) alfaxalone will be a useful option for in-tank immobilization procedures of large marine teleost species thereby facilitating examination, transport and other procedures. Alfaxalone was administered i.m. to permits at 5 mg/kg (*n*= 8), 7 mg/kg (*n*= 1) and 10 mg/kg (*n*= 2) doses and to schoolmasters at a 5 mg/kg dose (*n*= 4). Response to stimuli and opercular rates were measured every 5 min. The majority of permits and schoolmasters experienced mild, smooth sedation at the 5 mg/kg dose. All permits experienced prolonged excitement phases and duration of effect at the 7 and 10 mg/kg doses. Thus, these doses are not recommended in permits and similar species.

**Key words:** Alfaxalone, *Lutjanus apodus*, marine teleost, sedation, *Trachinotus falcatus*

**LITERATURE CITED**


CHOLECALCIFEROL SUPPLEMENTATION IN CAPTIVE ASIAN ELEPHANTS  
(*Elephas maximus*)

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Abstract

A herd of Asian elephants (*Elephas maximus*) maintained in a northern temperate climate was documented by the authors to have severely low total serum vitamin D levels over a period of 1 yr. This is of concern, since vitamin D plays an essential role in normal calcium and phosphorus homeostasis and skeletal function. In addition, vitamin D also has been shown to have important functions in numerous other systems, including immune and reproductive systems.1,2 The goal of this study was to monitor the effectiveness of oral vitamin D3 supplementation for increasing serum 25(OH)D3 concentration in Asian elephants, and also to observe its effects on other parameters associated with calcium and vitamin D metabolism.

Six healthy adult Asian elephants, were given 318 IU/kg of cholecalciferol orally once weekly for 16 wk. Every 4 wk during this period, serum was analyzed for 25(OH)D2/D3, 1,25(OH)2/D3, 24,25(OH)2/D3, parathyroid hormone, ionized calcium, and minerals (calcium, magnesium, phosphorus). Prior to the beginning of supplementation, the serum 25(OH)D3 level in all of the elephants was nondetectable. In all of the elephants, supplementation with cholecalciferol resulted in a steady rise in 25(OH)D3 during the treatment period to concentrations considered acceptable and safe. A positive correlation of 24,25(OH)2/D3 with serum 25(OH)D3 concentration was observed. This study provides new and valuable information regarding the kinetics of vitamin D metabolism in the Asian elephant in response to cholecalciferol supplementation, and provides a basis for further investigations exploring vitamin D metabolism, and refining nutrient requirements in this species.

Key words: Asian elephant, cholecalciferol, *Elephas maximus*, supplement, vitamin D

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The authors would like to thank the staff of the Rosamond Gifford Zoo, including the elephant animal care team and Sue Faso, for assistance with sample collection and processing.

LITERATURE CITED


NEW WORLD SCREWWORM (Cochliomyia hominivorax) OUTBREAK AND TREATMENT IN ENDANGERED KEY DEER (Odocoileus virginianus clavium)

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Abstract

Infesting only living tissue, the New World screwworm (NWS), Cochliomyia hominivorax, differs from other North American blow flies, which feed on decaying tissues.2 With the female capable of depositing up to 450 eggs onto a wound as small as a tick bite, NWS myiasis can quickly become life threatening and has resulted in significant economic losses in domestic livestock.2 NWS infestations capable of sustained reproduction were eradicated in the United States in 1966, with the last reported case in 1982.1,2 In September 2016, NWS infestation was documented in an endangered Key deer (Odocoileus virginianus clavium) on Big Pine Key, Florida. Primary eradication measures included biologic control through sterile fly release. Additional efforts to reduce the impact on Key deer and reduce fly numbers included euthanasia of severely affected deer, topical and oral administration of doramectin to free-roaming deer, treatment of mild to moderate infestations, and gamete preservation. Treatment included larvae removal, wound care, topical coumaphos, injectable doramectin, antibiotics, and marking for followup. Of 25 animals assessed by veterinarians, six were euthanized, 17 were treated, and two were monitored without handling. Two animals later died due to trauma and one animal required re-treatment. Treated animals were identified at 3 mo post assessment with clinical resolution or improvement. While biologic control measures were most effective in reducing fly numbers and ending the outbreak, treatment proved effective for mild to moderate infestations and resulted in preservation of individuals, reduction in fertile flies, and improvement in community support for the eradication program.

Key words: Cochliomyia hominivorax, infectious disease, Key deer, Odocoileus virginianus clavium, screwworm

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The authors would like to acknowledge the staff at the US Department of Agriculture/Animal and Plant Health Inspection Service, the US Fish and Wildlife Service, the US National Parks Service, the Florida Fish and Wildlife Conservation Commission, the Florida Department of Agriculture and Consumer Services, the Screwworm Barrier Maintenance Program in Panama, and the community of the Florida Keys for their roles in this multi-agency and community-assisted eradication program.

LITERATURE CITED

USE OF PALMAR DIGITAL NEURECTOMY TO MANAGE CHRONIC LAMENESS IN AN ADULT RETICULATED GIRAFFE (Giraffa camelopardalis reticulata)

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Abstract

An 11-yr-old female giraffe (Giraffa camelopardalis reticulata) with a history of excessive hoof overgrowth and white line disease has been managed for the past 4 yr with nonsteroidal anti-inflammatory drugs (NSAID; flunixin meglamine: 1-2 mg/kg p.o., s.i.d.-b.i.d. then firocoxib: 0.157-0.5 mg/kg p.o., s.i.d.-b.i.d.), analgesics (gabapentin: 3 mg/kg p.o., b.i.d.), nutraceuticals (FlexMax [glucosamine/chondroitin sulfate, hylauronic acid]: 1 oz p.o., s.i.d. and Conquer (hylauronic acid): 0.007 ml/kg) and topical foot soaks (ZnSO₄ and New-Hoof Concentrate 7.6% diluted) in addition to biannual immobilizations for aggressive hoof trims. Even with this intensive therapy, this giraffe maintained a level of lameness of 3 to 4 (using Dallas Zoo lameness scale, Table 1) and was unable to go on exhibit with the entire herd. Concerned for this animal’s quality of life, the decision was made to perform a palmar digital neurectomy to improve mobility and possibly decrease (or eliminate) the need for NSAIDs or analgesic therapy. Palmar digital neurectomy is commonly used in horses to relieve chronic pain; however, it is an uncommon procedure in bovids though the enervation of each claw is similar to that of the equid hoof. In this case, a unilateral palmar digital neurectomy was performed using standard techniques on the right foreleg, which was the most severely affected. Within 24 hr, staff reported that this animal’s lameness had improved from a 4 to a 2, and it was able to now circle to the right easily. Prior to neurectomy this giraffe would often avoid turning to the right if possible. This giraffe is currently maintaining a 2 to 3 level of lameness and the hope is to perform a similar neurectomy on the left foreleg at the next hoof trim which may normalize mobility completely. The analgesics have been discontinued and the NSAID administration dosage is currently being decreased. This appears to be the first time this procedure has been attempted in an adult giraffe and demonstrates a successful management option for those animals with unresolvable, chronic lameness of digital origin.¹⁴

Key words: Chronic lameness, Giraffa camelopardalis reticulata, giraffe, palmar digital neurectomy

LITERATURE CITED


Table 1. Dallas Zoo lameness scoring system.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
<th>Rest Analysis</th>
<th>Gait Analysis</th>
<th>Movement in Pen/Exhibit</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>Sound</td>
<td>Puts even weight on all limbs</td>
<td>Normal gait</td>
<td>Moves around normally</td>
<td>No lameness</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Inconsistently lame</td>
<td>Normal</td>
<td>Subtle occasional limp</td>
<td>Freely moves with occasional lameness</td>
<td>Normal posture at rest, occasional limp when moving</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Slight head nod or quick gait</td>
<td>Moves around freely but with constant mild lameness</td>
<td>Occasional change in posture, may need to exercise to see lameness</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Mildly lame</td>
<td>Occasionally shifts weight or lifts lame leg</td>
<td>Slight head nod or quick gait that is consistent and obvious, short striding Grade 3 signs, plus 3 legged lame at times</td>
<td>Moderately reluctant to move, difficulty turning and making frequent stops</td>
<td>Abnormal posture, deliberate steps one at a time, may have difficulty achieving urinating posture</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Moderately lame</td>
<td>Shifts weight off of leg, toe touching obvious</td>
<td>Grade 4 signs, Inability to or very reluctant to move</td>
<td>Inability to or very reluctant to move</td>
<td>Severely poor quality of life, behavior and well-being are affected, isolation from herd</td>
</tr>
<tr>
<td>Grade 4</td>
<td>Very lame</td>
<td>Holds leg off ground at rest</td>
<td>Grade 4 signs, Inability to or very reluctant to move</td>
<td>Moderately reluctant to move, difficulty turning and making frequent stops Inability to or very reluctant to move</td>
<td>Abnormal posture, deliberate steps one at a time, may have difficulty achieving urinating posture</td>
</tr>
<tr>
<td>Grade 5</td>
<td>Severely lame</td>
<td>Prefers to lay down, depressed</td>
<td>Grade 4 signs, Inability to or very reluctant to move</td>
<td>Inability to or very reluctant to move</td>
<td>Severely poor quality of life, behavior and well-being are affected, isolation from herd</td>
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</tbody>
</table>
IS IT TRUE THAT ELEPHANTS DON’T GET CANCER? LESSONS IN USING MORTALITY DATA

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Abstract

A recently published scientific study examined cancer prevalence across multiple species of mammals in support of the hypothesis that elephants possess genetic mechanisms conferring resistance to the development of cancer.1 The study included cancer prevalence data abstracted from a summary of San Diego Zoo necropsy findings for several species housed in its facility from 1964 to 1978.2 The study used mortality data for elephants from an online database (Elephant Encyclopedia: www.elephant.se, accessed May 2017) compiled by self-report for a lay audience. The approach used for this study illustrates several potential pitfalls in the use of retrospective mortality data from zoo collections. To further examine findings, the team reabstracted data from Griner and added new data from the San Diego Zoo’s database of mortality records with attention to important interpretation details that were not included in the original report.2,3 The study team calculated cancer prevalence for several species, and compared new estimates to those from the original report. Findings from the limited sample size show that elephants necropsied at the San Diego Zoo from 1987-2015 did acquire cancer in proportions similar to humans: 8/12 were diagnosed with neoplasia, one-third (4/12) had malignant neoplasia, and two geriatric elephants died of neoplasia. These refined cancer prevalence estimates across all of the evaluated species illustrate the biases that can be introduced in mortality reviews based on insufficient case definitions, inadequate identification of the at-risk population, temporal confounding, unreliable sources of data, and/or interpretative details important for drawing conclusions.

Key words: Cancer, elephant, mortality, mortality review

ACKNOWLEDGMENTS

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LITERATURE CITED


SERUM IMMUNOGLOBULIN E MEASUREMENTS AND CLINICAL RESPONSE TO ALLERGEN-SPECIFIC IMMUNOTHERAPY (ASIT) IN ASIAN ELEPHANTS (Elephas maximus)

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Abstract

Baseline immunoglobulin E levels in elephant species are currently limited, and allergies are infrequently diagnosed. In this study, serum samples from three African (Loxodonta africana) and eight Asian (Elephas maximus) elephants were submitted for measurement of allergen-specific immunoglobulin E (IgE) for 91 environmental and/or 29 food allergens. High affinity IgE allercpt analysis was performed. Two animals with increased IgE levels and exhibiting clinical signs were treated with allergen-specific immunotherapy (ASIT). Case 1: One female elephant exhibited severe pruritus from April to August and a reluctance to exit the barn for these months for approximately 3 yr prior to initial IgE measurement. The animal exhibited elevated IgE levels ranging from 11 to 128 HERBU, with standard of measurement based on degree of fluorescence. Positivity was reported to 18.6% of grass, weed, and tree species and insect hypersensitivity to three species: Culicoides, deer, and horse flies. In addition to implementing environmental controls, the elephant was treated with oral antihistamines and weekly ASIT beginning 06/2015. ASIT injections were administered per manufacturer guidelines (HESKA, Loveland, Colorado, 80538), in increasing concentration, quantity, and frequency until a maintenance dose was reached. The elephant receives 1.5 ml/wk from April to August, in combination with antihistamines and 1 ml every 2 wk without antihistamines for the remainder of the year. The animal’s SCORAD indices decreased from baseline progressively each spring, with a 15% reduction in 2015 and a 44% reduction in 2016. Less severe to no symptoms were exhibited during fall and winter. Case 2: One female elephant with mild, intermittent gastrointestinal discomfort was found to have significantly elevated IgE to 85.7% of environmental allergens tested, with IgE levels greater than 600 for the majority of grasses, weeds, trees, mites, and insects tested. The elephant displayed an abnormal hypersensitivity response to 24/29 foodstuffs. A major dietary exclusion trial was initiated. Concurrently, oral ASIT was initiated in 08/2016 and was administered at 1 ml b.i.d. sublingually. It is likely that total allergen load contributed to a summation effect, increasing the overall severity of symptoms, which included cuticle rubbing, inconsistent consumption of routine foodstuffs, and chronic, seasonal urticaria with variable sized wheals. The SCORAD indices have decreased 18% from baseline in the past year. Management of the animal’s diet has continued to prove challenging and is a work in progress. Accuracy and/or predictability of in vitro serum testing for elephant atopic dermatitis and hypersensitivity response to normal foodstuffs cannot be determined by this study; further study is needed. This study did not show strong correlation between degree of IgE elevation and severity of clinical signs; however, IgE levels decreased 61% overall in animals that were treated with ASIT, and severity of type 1 hypersensitivity reaction related clinical signs were reduced, despite a variable reduction in measured IgE levels. Measurement of serum IgE may offer insight into diagnosis and treatment of elephants displaying atopy or abdominal discomfort.
**Key words:** Allergy, *Elephas maximus*, immunoglobulin, immunotherapy

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The authors would like to acknowledge Heska Corporation for measuring IgE levels, and the elephant caregivers, whose dedicated daily care makes individualized veterinary care possible.

**LITERATURE CITED**


SUCCESSFUL MANAGEMENT AND RESOLUTION OF A SEVERE NECROTIZING DERMATOPATHY IN A GOLDEN-HEADED LION TAMARIN (*Leontopithecus chrysomelas*)

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Abstract

An 8-yr-old female golden-headed lion tamarin (*Leontopithecus chrysomelas*) with a prior history of metabolic bone disease and cholelithiasis presented with a full thickness, 3 cm × 2 cm ulceration on the right lateral thorax and abdomen, with multiple furuncles at the wound edges and on the lateral aspect of the right arm. Unknown trauma with secondary infection was the most likely etiology. Histopathology indicated an ulcerative dermatitis with colonies of coccoid bacteria; severe, necrotizing cellulitis; and pyogranulomatous furunculosis. An aerobic culture grew *Staphylococcus aureus* that was sensitive to most common antibiotics. Initial treatments consisted of amoxicillin/clavulanic acid (Pfizer, New York, New York 10017 USA), ciprofloxacin (Lupin Limited, Baltimore, Maryland 21202 USA), and meloxicam (Boehringer Ingelheim, St. Joseph, Missouri 64506 USA), and evaluations were performed every 3-5 days for topical treatments with dilute chlorhexidine (Agrilabs, St. Joseph, Missouri 64505 USA) and/or betadine (Purdue Products, Stamford, Connecticut 06901 USA), and topical silver sulfadiazine (SSD 1%, Ascend Laboratories, LLC, Parsippany, NJ 07054). Within 2 wk, the initial wounds had contracted, but thick, raised, brown plaques had developed. At 4 wk, the plaques had spread over the right side of the body (7.6 cm × 3.5 cm). Repeat skin biopsies showed severe necrosis and neutrophilic infiltration with mixed bacterial infection of the epidermis and dermis, and macrophage-engulfed granules that were suspected to be related to ointments. Possible etiologies for the extensive response included a staphylococcal toxin reaction, a reaction to systemic or topical medications (SSD, or betadine), or an underlying immune pathology leading to a pyoderma vegetans-type lesion. Topical chlorhexidine and oral amoxicillin/clavulanic acid and ciprofloxacin were continued, clindamycin (Greenstone Brand, Peapack, New Jersey 07977 USA) was added due to its anti-toxin effects, and an anti-inflammatory course of prednisone (Boehringer Ingelheim, St. Joseph, Missouri 64506 USA) was administered and tapered. At week 7, the plaques had spread to cover approximately 40% of the dorsum and the right side of the thorax and abdomen. Repeat biopsies showed severe ulceration bordered by severe hyperplastic epidermis covered with a crust of exudate, degenerate neutrophils and bacteria. Chemical debridement with hydrogen peroxide (*H₂O₂*) was initiated, consisting of covering the area in *H₂O₂*-soaked gauze for 5-10 min, and then using *H₂O₂*-soaked cotton tipped applicators to mechanically debride the plaques. This procedure was repeated every 3-5 days for the first 2 wk, and immediate improvement and epithelialization of the borders of the affected tissue were seen. Repeat debridements were performed every 2-4 wk for 4 mo. During this time, oral clindamycin was the only oral treatment administered. Complete resolution of the lesion was seen 4 mo after *H₂O₂* debridement was initiated, with limited scarring, and normal hair coverage. This case highlights the importance of chemical debridement in management of necrotizing dermatopathies.
Key words: Chemical debridement, golden-headed lion tamarin, *Leontopithecus chrysomelas*, necrotizing dermatopathy

LITERATURE CITED


DISSEMINATED POLYSYSTEMIC LEIOMYOSARCOMA WITH INTRACRANIAL METASTASIS IN A MALE AFRICAN LION (Panthera leo)

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Abstract

A 14-yr-old male African lion (Panthera leo) presented with lethargy and serous epiphora from the right eye. Clinical signs progressed over the next 5 wk to include right unilateral enophthalmia, ptosis, mydriasis, atrophied temporal and masseter muscles, and intermittent epistaxis. Complete blood count and select sera chemistries did not reveal abnormalities. Rickettsial disease assays were negative. The 2M antibody test for masticatory muscle myositis was negative. Affected skeletal muscle biopsies revealed mild myositis and rhabdomyolysis. The lion became refractory to treatment and was humanely euthanized. Postmortem magnetic resonance imaging (MRI) of the head revealed an intracranial mass in the region of the right oculomotor and trigeminal nerves. Necropsy revealed masses in numerous organs. The intracranial mass was closely associated with the right oculomotor and trigeminal nerves. Histologic exam identified a disseminated spindle cell sarcoma. Immunohistochemical labeling was positive for smooth muscle actin, and the tumor was diagnosed as a metastatic leiomyosarcoma of undetermined tissue origin. Clinical signs were attributed to multisystem metastatic lesions rather than the primary tumor.

Key words: Atrophy, cranial nerves, immunohistochemistry, leiomyosarcoma, metastatic, Panthera leo

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The authors would like to thank the animal care staff at the Kansas City Zoo for their care of the lion, Roy Brown of Histology Consulting Service for slide preparation, and Cathy Minogue of Northwest ZooPath for data retrieval.
LUMBOSACRAL DISEASE IN A CAPYBARA (*Hydrochoerus hydrochaeris*)

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Abstract

Lumbosacral disease is not commonly described in rodents and was diagnosed in a 13-yr-old capybara (*Hydrochoerus hydrochaeris*). The animal presented with a persistent penile prolapse unresponsive to chemical castration, hindlimb lameness, progressive ataxia, and a loss of bladder control. Its clinical condition had progressively worsened over multiple years with only marginal improvement on various treatments, and a decision was made to euthanize the animal. Computed tomography was performed postmortem to evaluate the utility of this diagnostic tool in this species and to have a baseline for comparison in the future for its conspecific of a similar age. The imaging study revealed degenerative changes of the lumbosacral spine with instability of the L6-S1 disc space and likely impingement of the spinal nerve roots as a result of mineral foci in the intervertebral foramina. The necropsy confirmed the presence of a severe, chronic degenerative intervertebral disc disease at the lumbosacral junction with ankylosing spondylosis, consistent with the diagnosis of lumbosacral disease seen in other mammals. In summary, large rodents appear susceptible to the same degenerative articular changes in the spine as domestic mammals, and lumbosacral disease should be included in the list of differential diagnoses of large rodents presenting with similar clinical signs of neurologic impairment.

**Key words:** Capybara, computed tomography, *Hydrochoerus hydrochaeris*, intervertebral disc disease, lumbosacral disease
OVIDUCT MORPHOLOGY AND PROLACTIN RECEPTOR EXPRESSION IN NORTH AMERICAN RIVER OTTERS (Lontra canadensis) AND ASIAN SMALL CLAWED OTTERS (Aonyx cinerea)

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Abstract

Otter populations worldwide have been declining due to environmental pressures and habitat loss. Gaps in knowledge of their reproductive physiology have slowed captive breeding efforts. The North American river otter (NARO), Lontra canadensis, and the Asian small-clawed otter (ASCO), Aonyx cinerea, have two distinct reproductive cycles. The NARO is monoestrous and seasonal with a marked embryonic diapause; whereas, the ASCO is polyestrous and nonseasonal and lacks delayed implantation. There is little information available on the role of the oviduct in diapause or mustelid reproductive physiology. It was hypothesized that the NARO and ASCO would differ in oviduct length and in uterine and ovarian prolactin receptor expression. Oviducts from archived tracts held by the Reproductive Health Surveillance Program from both species (ASCO, n = 14; NARO, n = 5) were dissected, the length measured and compared. The oviducts were then trimmed into cross-sectional segments and processed for comparison of histomorphology. The uterus and ovaries from a subset were processed and labeled with antibodies for prolactin (PRL) receptor. The stain intensity was scored at four locations (uterine epithelium, uterine glandular epithelium, ovarian parenchyma, and ovarian follicular structures) and compared. Findings were: 1) NARO oviducts were significantly longer than ASCO oviducts (P = 0.0085), 2) PRL receptor antibodies successfully labeled otter reproductive tissues, and 3) there was no significant difference in PRL receptor expression between NARO and ASCO reproductive tissues on preliminary analysis. Knowledge of the reproductive tract, in particular physiology of diapause, allows for improved reproductive interventions in these threatened species.

Key words: Aonyx cinerea, diapause, Lontra canadensis, oviduct, prolactin, reproductive physiology

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BILATERAL ALVEOLAR OSTEITIS DUE TO TRAUMATIC AVULSION OF THE MAXILLARY CANINES IN A SUMATRAN ORANGUTAN (Pongo abelii)

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Abstract

A 31-yr-old female Sumatran orangutan (Pongo abelii) was presented for evaluation of bilateral alveolar osteitis following traumatic avulsion of both maxillary canines during transport between zoological institutions. The patient was observed self-traumatizing the wounds repeatedly using both fingers and pieces of bamboo. Due to management and quarantine concerns, repair was attempted approximately 3 wk following the initial injury; medical management with antibiotics and nonsteroidal anti-inflammatory drugs was instituted with mixed success prior to this. At time of the surgery, the teeth were absent with no evidence of retained roots, but severe gingival recession was noted. A single-layer mucoperiosteal gingival flap was performed at each site following debridement of granulation tissue and necrotic alveolar bone. Postoperative healing had no complications, and followup 4 mo later showed complete resolution. Wound healing was potentially challenging due to the species; however, close cooperation of the veterinary staff and zookeepers allowed a comprehensive postoperative recovery plan to be implemented that included behavioral enrichment, positive reinforcement, and frequent conscious rechecks.

Key words: Behavioral enrichment, gingival flap, oral trauma, orangutan, Pongo abelii, tooth avulsion

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The authors thank Ms. Sarah Schwenzer, LVT and Ms. Jennifer Stahl for assistance in developing the postoperative care plan for this patient.
INDEX

25-hydroxyvitamin D, 112

[A]
abomasum, 155, 156
Acinonyx jubatus, 81, 87, 88, 89, 176
Acropora cervicornis, 38, 42
acute phase response, 172, 176
adenocarcinoma, 28
adenovirus, 92, 120, 121, 171, 183, 197
advanced imaging, 162
African elephant, 66, 109, 112
African lion, 211
air sac, 27
alfaxalone, 4, 5, 6, 7, 10, 34, 43, 44, 45, 46, 181, 184, 200
alfaxalone, 4, 6, 8, 10, 43, 181, 184, 200
Alfaxan, 4, 6, 7, 44, 46, 200
alligators, 13
allostatic load, 148, 149
alpha herpes virus, 126
amantadine, 164
Amazona ventralis, 29
Ambystoma platinuem, 115
American black bears, 3
amphibian, 74, 75, 115, 179
amyloid, 100, 101, 124, 129, 172, 176, 198, 199
amyloidosis, 87, 124, 129, 175, 177
analgesia, 37, 164
analgesic, 6, 107, 146, 164, 170, 204
anatomy, 27, 59, 132, 185
Anaxyrus houstonensis, 75, 78, 184
anesthesia, 1, 2, 3, 4, 6, 7, 10, 11, 27, 29, 36, 37, 38, 39, 43, 44, 45, 46, 48, 64, 83, 96, 100, 125, 128, 144, 154, 157, 180, 181, 184, 200
anesthesia maintenance, 2
anesthetic, 1, 2, 3, 4, 6, 7, 32, 34, 36, 43, 44, 45, 46, 64, 180, 184, 200
anesthetic monitoring, 1
Animal and Plant Health Inspection Service, 15, 202
animal health, 140, 151, 152, 153, 189
animal welfare, 146, 153, 189, 195
anteater, 96
antelope, 125, 126, 129, 175
Antennariidae, 59
Antilocapra americana, 129
antimicrobials, 30, 91, 161, 168
appendicular fractures, 17
aquariums, 30, 32, 59, 189
Arctocephalus australis, 62, 63
arthitis, 190, 193
Asian elephant, 100, 111, 201, 207
aspergillosis, 27, 57, 58, 174, 176, 177, 197
assisted breeding, 107
Ateles geoffroyi, 51
atrioventricular block, 159
avian bornavirus, 12, 14
avian infectious diseases, 12, 15
avian influenza, 12, 13, 14, 15
avocado, 55, 56
aye-aye, 55
azaperone, 8, 10
[B]
bald eagle, 164
barium, 29
bearded dragons, 174, 182, 185
bears, 3, 187, 188, 190, 191, 192, 193, 194
behavior, 26, 36, 144, 157, 158, 187, 188, 194, 205
bighorn sheep, 8
bird, 12, 13, 14, 15, 16, 23, 26, 97, 160
birds of prey, 14, 17
black rhinoceros, 103, 132, 133, 135, 136, 137, 138, 139, 140, 141
black-footed ferret, 85
Blaptica dubia, 43, 44
blood pressure, 1, 3, 10, 11, 155
boar, 107, 108
body condition scoring, 125
body weight, 64, 196
bone marrow, 109, 136, 167, 168, 169
bongo, 124
bonobo, 2
breeding, 23, 37, 75, 78, 107, 132, 136, 138, 144, 150, 213
Bubo scandiacus, 19, 20
Buceros rhinoceros, 160
<table>
<thead>
<tr>
<th><strong>a</strong></th>
<th><strong>b</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>buprenorphine</strong>, 24, 182</td>
<td><strong>crickets</strong>, 179</td>
</tr>
<tr>
<td><strong>Buteo jamaicensis</strong>, 24</td>
<td><strong>crop</strong>, 22, 29, 97</td>
</tr>
<tr>
<td>butorphanol, 29, 107, 157</td>
<td>cryopreservation, 81, 82, 167</td>
</tr>
<tr>
<td>calcium, 40, 111, 112, 169, 193, 201</td>
<td>cyclobenzaprine, 187, 188</td>
</tr>
<tr>
<td>callitrichid, 54</td>
<td>cyclosporine, 128, 210</td>
</tr>
<tr>
<td>Cameroon red tarantula, 40</td>
<td>cyst, 162, 169, 170</td>
</tr>
<tr>
<td>cancer, 206</td>
<td><strong>[C]</strong></td>
</tr>
<tr>
<td><em>Candida</em>, 97</td>
<td><strong>[D]</strong></td>
</tr>
<tr>
<td>canine distemper, 94, 95</td>
<td>darting, 154</td>
</tr>
<tr>
<td>capture, 8, 180</td>
<td>data, 11, 24, 30, 31, 37, 52, 59, 64, 68, 70, 74, 79, 83, 85, 87, 89, 95, 97, 122, 128, 134, 135, 146, 166, 169, 171, 174, 185, 187, 189, 195, 206, 211</td>
</tr>
<tr>
<td>capybara, 175, 212</td>
<td>database, 15, 51, 72, 206</td>
</tr>
<tr>
<td>cardiac problems, 136, 159</td>
<td><em>Daubentonia madagascariensis</em>, 55</td>
</tr>
<tr>
<td>cardiomyopathy, 160</td>
<td>decision making, 142</td>
</tr>
<tr>
<td>caregiver placebo effect, 146</td>
<td>deer, 10, 103, 202, 207</td>
</tr>
<tr>
<td>cell count, 40, 124, 158, 166, 173, 183</td>
<td>dental disease, 155</td>
</tr>
<tr>
<td>chameleons, 92</td>
<td>dermatitis, 97, 113, 114, 207, 208, 209</td>
</tr>
<tr>
<td>cheetah, 81, 88, 175, 176</td>
<td>dermatopathy, 209, 210</td>
</tr>
<tr>
<td>chelonian, 118, 183</td>
<td>dexamethomidine, 6, 7, 184</td>
</tr>
<tr>
<td>chemical castration, 212</td>
<td>diagnostic imaging, 66, 83</td>
</tr>
<tr>
<td>chemical restraint, 29</td>
<td>diazepam, 157, 180</td>
</tr>
<tr>
<td><em>Chiloscyllium plagiosum</em>, 67</td>
<td>diet, 23, 67, 83, 111, 130, 131, 150, 155, 193, 207</td>
</tr>
<tr>
<td>chimpanzee, 49, 50, 144</td>
<td>dik-dik, 158</td>
</tr>
<tr>
<td>cholecalciferol, 112, 201</td>
<td>disease, 12, 13, 14, 15, 16, 18, 19, 21, 22, 26, 27, 30, 31, 36, 38, 54, 62, 67, 68, 69, 70, 72, 74, 75, 76, 79, 80, 85, 87, 88, 92, 94, 95, 96, 97, 98, 100, 102, 111, 113, 114, 115, 117, 118, 120, 128, 132, 133, 134, 138, 139, 140, 146, 148, 149, 150, 152, 155, 158, 159, 165, 167, 170, 173, 175, 177, 185, 186, 189, 193, 197, 202, 204, 209, 211, 212</td>
</tr>
<tr>
<td>cholelithiasis, 209</td>
<td>distemper virus, 94, 95</td>
</tr>
<tr>
<td><em>Choleopus didactylus</em>, 94</td>
<td>dolphins, 57, 60, 61, 128, 175</td>
</tr>
<tr>
<td><em>Chrysocyon brachyurus</em>, 161</td>
<td><strong>[E]</strong></td>
</tr>
<tr>
<td>chytrid, 74, 115</td>
<td>Eastern box turtle, 118, 120, 121</td>
</tr>
<tr>
<td>chytridiomycosis, 74, 115</td>
<td>education, 154</td>
</tr>
<tr>
<td>claw disorders, 190</td>
<td>elasmobranch, 67</td>
</tr>
<tr>
<td>clinical pathology, 70, 83, 174, 183</td>
<td>electrocardiogram, 60</td>
</tr>
<tr>
<td><em>Clostridium difficile</em>, 51</td>
<td>electrolyte reference values, 40</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em>, 22, 23, 105, 150</td>
<td>elephant endotheliotropic herpesvirus, 177</td>
</tr>
<tr>
<td>coagulation, 40</td>
<td>elephant seals, 175, 198, 199</td>
</tr>
<tr>
<td><em>Coccidioides immitis</em>, 158</td>
<td>elephant seals, 198</td>
</tr>
<tr>
<td>coccidiosis, 85</td>
<td>elephant training, 99</td>
</tr>
<tr>
<td><em>Cochliomyia hominivorax</em>, 202</td>
<td><em>Elephas maximus</em>, 66, 99, 100, 101, 111, 112, 177, 201, 207, 208</td>
</tr>
<tr>
<td>cockatiels, 29, 97</td>
<td></td>
</tr>
</tbody>
</table>
ELISA assay, 57
embryo development, 42
emerging diseases, 70
emerging infectious diseases, 115, 183
Emydoidea blandingii, 118, 119
encephalitis, 13, 14, 127
end-of-life care, 142
endoscopic visualization, 18
endoscopy, 18, 83
enteritis, 22, 23, 85, 150, 173
enterotoxemia, 105
Equus caballus, 130, 131
Equus grevyi, 126
Erpeton tentaculatum, 71
estradiol, 130, 131
estrous cycle, 130, 131
etorphine, 107
Eudyptula minor, 27
exercise, 144, 192, 205
eyelid agenesis, 86
eyelid coloboma, 86
[F]
F10 veterinary disinfectant, 74
felid, 81, 89
felids, 81, 82, 89, 90, 96
feline infectious peritonitis, 173
Felis concolor, 83
fentanyl, 2, 107
fish, 36, 157, 175, 179, 193, 200
fluoxetine, 188
food-producing animals, 91
foreign animal disease, 98
fracture, 17, 143, 157, 169, 204
frogfish, 59
frogs, 116
fungal infections, 57
fungus, 59, 71, 74, 115, 197
fur seal, 62, 63
[G]
gabapentin, 164, 204
gastrointestinal disease, 22, 83, 97, 150
gastrointestinal transit times, 29
Geochelone, 180
giant anteaters, 96
Giraffa camelopardalis, 1, 127, 155, 204, 205
giraffe, 1, 126, 127, 155, 204, 205
glaucoma, 48
glomerular filtration, 68, 87, 88, 185, 186
gopher tortoise, 72, 76
Gopherus polyphemus, 72, 76
gorilla, 2, 48, 49, 52, 53, 144, 148, 149
Gorilla gorilla gorilla, 48, 49, 144, 148, 149
great ape, 2, 49, 158
green iguana, 6, 186
green turtle, 73
Gromphadorhina portentosa, 43, 44
gross lesion recognition, 165
guaifenesin, 3
guanaco, 162
guide, 11, 38, 142, 180
guidelines, 40, 139, 159, 207
[H]
half-life, 24, 89, 170, 173
hand-rearing, 52
hawks, 15, 24
health assessment, 27, 40, 115, 120, 172, 175
health parameters, 183
heart, 6, 8, 10, 55, 60, 79, 80, 159, 160, 181, 184, 196
heart disease, 80
hematology, 18, 70, 72, 73, 133, 150, 183
hemolymph cytology, 40
hemoparasite, 19
herpesvirus, 62, 63, 92, 93, 118, 119, 120, 121, 126, 127, 177, 183
heterophil, 76, 120
hoofstock, 102, 125, 158
hoofstock, 125
hornbill, 160
horse, 167, 168, 169, 171, 199, 207
horseshoe crabs, 30, 34
Houston toad, 75, 78, 184
hummingbirds, 197
humoral immune response, 26
Hydrochoerus hydrochaeris, 212
hydromorphone, 7, 25, 182
hyperthyroidism, 162
hyperviscosity, 79
hypothermia, 43, 79
hypothyroidism, 49, 50
Hysterocrates gigas, 40
[I]
imaging, 27, 66, 83, 84, 109, 150, 160, 162, 165, 166, 211, 212
immobilization, 8, 10, 37, 46, 65, 107, 180, 181, 200
immunotherapy, 207, 208
implant, 128, 169
*Incilius alvarius*, 181
*Indotestudo elongata*, 178
infertility, 123, 130, 131
inflammation, 72, 73, 76, 79, 89, 100, 128, 129, 162, 167, 169, 170, 173, 175, 190, 198
injection, 6, 8, 10, 13, 37, 38, 43, 45, 46, 89, 109, 162, 168, 169, 170, 174, 181, 184, 185, 196, 200
insulin, 169, 170
interleukin receptor antagonist protein, 100, 101
intervention, 26, 52, 132, 136
intravenous anesthesia, 2
invertebrate feed, 179
iodine, 31, 67, 162, 210
iodine supplementation, 67
iohexol, 29, 185, 186
IRAP, 100, 170
iron overload, 132, 133, 135, 137, 138, 139, 140
isoflurane, 2, 3, 7, 11, 37, 40, 44, 200

J

jellyfish, 30, 31

K

ketamine, 3, 8, 37, 46, 81, 180
kidney, 68, 69, 79, 87, 88, 92, 115, 124, 185, 186
kidney disease, 68, 69, 87, 88

L

*Lama guanicoe*, 162
lameness, 109, 143, 144, 146, 166, 168, 204, 205, 212
laser, 48, 144
leadership, 152, 153
leiomyosarcoma, 211
lemur, 196
*Leontopithecus chrysomelas*, 209, 210
lepidopterism, 113, 114
*Lepilemur sahamalaza*, 196
lesion recognition, 165
leukopenia, 72
lion, 62, 63, 81, 89, 113, 164, 209, 210, 211
liver biopsy, 18

Loxdonta africana, 109
lumbosacral disease, 212
*Lutjanus apodus*, 200

M

Madagascar hissing cockroach, 43
*Madouqa kirkii*, 158
manatee, 66, 68, 69, 175, 176
maned wolf, 161
*Manis tricuspis*, 150
mare, 114, 130, 131, 199
medetomidine, 3, 8, 10, 81
*Megadyptes antipodes*, 21
meloxicam, 22, 89, 90, 126, 146, 209
*Melursus ursinus*, 190
metronidazole, 51
microbial integrity, 4
microbiome, 51
midazolam, 29, 157
*Mirounga angustirostris*, 198
mission, 154
morbidity, 12, 17, 19, 26, 55, 59, 67, 75, 85, 92, 117, 118, 121, 124, 132, 136, 137, 138, 150
morphine, 37, 46
mortality, 12, 17, 19, 21, 22, 26, 44, 45, 52, 55, 59, 64, 65, 67, 71, 75, 79, 85, 92, 111, 113, 118, 120, 121, 124, 132, 136, 137, 148, 189, 194, 197, 206
moxidectin, 125
MS-222, 36, 184
*Mustela nigripes*, 85
mycobacteriosis, 27, 75, 197
mycobacteriosis, 75
*Mycobacterium orygis*, 102, 103
*Myrmecophaga tridactyla*, 96

N

neonatal treatment, 22
neoplasia, 78, 79, 122, 161, 173, 206
nitroprusside, 10
nonhuman primate, 51, 148, 175, 192
nonsteroidal anti-inflammatory, 204, 214
Northern elephant seals, 198, 199
nutrition, 67, 142, 193, 194
*Nymphicus hollandicus*, 29, 97

O

Odocoileus virginianus, 10, 103, 202
opioids, 107, 164
orange-spotted cockroach, 43, 44
orangutan, 2, 49, 50, 164, 175, 214
organizational influence, 151, 152, 153
orthopedics, 166
osteitis, 204, 214
osteoarthritis, 89, 90, 100, 109, 110, 146, 164, 168, 170
osteitis, 100, 109, 164
Otaria byronia, 62
otariids, 62, 64, 113
otters, 213
ovariohysterectomy, 89
oviduct, 213
Ovis canadensis, 8

[Pacemaker, 159
palmar digital neurectomy, 204
pangolin, 150
Panthera leo, 81, 89, 211
Panthera tigris, 11, 81, 89, 90
Parannizziopsis, 71
parasitic infections, 54
parrots, 29, 117, 174
pathogen prevalence, 21, 70
Pavo cristatus, 6
peafowl, 6
penguin, 21, 27, 28, 146, 164
peripheral nerve sheath, 78
Persea americana, 55, 56
pesticides, 36
pharmacokinetics, 6, 24, 30, 31, 37, 38, 89, 182
pharmacology, 7, 30, 45
pharmacology, 30
phosphorus, 111, 112, 193, 201
phytoestrogen, 130, 131
pigeons, 13, 14
pinnipeds, 62, 128
plasma biochemistry, 18, 186
plethysmographic variability index (PVI), 11
pneumonia, 75, 126, 129, 158, 173
Pogona vitticeps, 182, 185
poisoning, 55, 125
polar bear, 188, 192, 193
polymerase chain reaction, 14, 92, 105, 118
Pongo abelii, 49, 214
Pongo pygmaeus, 2, 49, 50
pregnancy, 66, 114, 150
pregnancy diagnosis, 66
preparedness, 98
primate, 55, 148, 196
prolactin, 66, 213
psittacine birds, 13, 18, 29
public health, 13, 14, 114
pulmonary hypertension, 160
puma, 81, 83
pyometra, 122, 123

[Q]
quality-of-life, 142
quarantine, 14, 189, 214

[R]
radiography, 18, 29
rainbow lorikeye, 26
ranavirus, 70, 92, 115, 120, 121, 183
ranavirus, 92, 115
red-tailed hawk, 24
regenerative medicine, 109, 166, 167, 171
rehabilitation, 17, 21, 64, 65, 66, 69, 120, 143, 145, 171, 176, 177
reintroduction, 52
renal, 68, 69, 87, 88, 113, 155, 185, 186
renal insufficiency, 68
renal portal system, 185
reproduction, 42, 52, 130, 202
reproductive tract, 122, 130, 213
reptiles, 70, 72, 73, 76, 77, 79, 118, 119, 174, 178, 179, 180, 182, 183, 185, 186, 200
research, 3, 6, 12, 21, 30, 32, 33, 34, 35, 40, 43, 82, 85, 87, 139, 146, 166, 168, 171, 180, 185, 188, 195
reticulated giraffe, 127, 155, 204, 205
rhinoceros, 102, 103, 104, 105, 107, 126, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 160
Rhinoceros unicornis, 102, 107
Rhynchopsitta pachyrhyncha, 117
risk, 14, 15, 17, 21, 26, 27, 43, 52, 64, 122, 126, 129, 148, 151, 152, 155, 167, 183, 189, 194, 206
risk factors, 17, 64, 122, 155
risk-based analysis, 189
river otter, 213
rockhopper penguin, 164
Rocky Mountain bighorn sheep, 8

[s]
salamander, 115
Salmonella, 26, 190
sarcocystosis, 117
Sarcophilus harrisii, 159
scarlet macaw, 28
screwworm, 202, 203
SDMA, 68, 87, 88
sea lions, 62, 63, 64, 65, 113
sea star, 35, 36, 38
seasonality, 115
Secure Zoo Strategy program, 98
semen, 81, 82, 107
semen banking, 81, 82
semen collection, 81, 107
semen cryopreservation, 82
serum acute phase proteins, 124
serum immunoglobulin E, 207
shark, 67, 175, 177
skeletal, 201, 211
skin, 55, 71, 78, 92, 96, 115, 157, 181, 209
sloth, 94
sloth bear, 190, 191
smartphone, 60
snake, 16, 70, 71, 158
snapper, 200
snow leopard, 86
snowy owl, 19, 20
spectacled bear, 193, 194
sperm, 42, 81, 82, 107, 108
sperm motility, 81, 107, 108
spider monkey, 51
springbok, 126
staghorn coral, 42
stem cells, 109, 166, 167, 168, 169, 171
stereotypic behavior, 187, 192
stress, 19, 44, 45, 55, 118, 139, 148, 149, 173, 175, 180, 187
Suidae, 122
supplementation, 49, 67, 112, 133, 134, 139, 201
surveillance, 14, 26, 54, 187
survey, 52, 88, 128
symmetric dimethylarginine, 68, 69, 87, 88

[t]
T3, 67, 162
T4, 49, 67, 162
tamarin, 209, 210
tapir, 128, 132, 135, 136, 139, 140
Tapirus indicus, 128
Tasmanian devils, 159
Tayassuidae, 122
teratoma, 161
terbinafine, 71
Terrapene carolina carolina, 92, 93, 118, 119, 120, 121, 177, 183
tiger, 11, 33, 38, 81, 90, 175, 194
tiletamine, 184, 196
tiletamine-zolazepam, 196
toads, 6, 74, 78, 181, 184
tortoise, 7, 72, 76, 121, 178, 180
toxicity, 55, 76, 125, 135, 139
toxicosis, 125
Trachinotus falcatus, 200
Tragelaphus eurycerus, 124
training, 60, 99, 142, 143, 152, 155
tramadol, 25
transport, 22, 60, 134, 136, 200, 214
transscleral micropulse laser therapy, 48
trauma, 68, 69, 173, 175, 197, 202, 209, 214
tricaine methanesulfonate, 184
Trichechus manatus latirostris, 66, 68, 69, 176, 177
Trichoglossus haematodus, 26
Trioceros melleri, 92
Tropidolaemus wagleri, 71
tuberculosis, 75, 102, 103, 104
tumors, 78
Tursiops truncatus, 57, 60, 61, 157
turtles, 70, 73, 93, 118, 119, 120, 121, 172, 174, 177, 182, 183, 186
Tymanuchus cupido attwateri, 22

[u]
ultrasound, 66, 130, 150, 161, 168, 170
Uncia uncia, 86
ungulates, 122
Ursus americanus, 3, 187

[v]
vaccination, 14, 26, 94, 105
vaccine, 13, 26, 94, 105
veterinary technician, 26, 59, 71, 87, 158, 160
virus, 12, 13, 14, 16, 20, 92, 94, 95, 96, 105, 115, 116, 118, 120, 121, 171, 175
vision, 32, 34, 48, 153
vitamin D, 111
vitrification, 81, 82
voriconazole, 71

[W]
walkway system, 146, 147
waterfowl, 14
welfare, 133, 146, 151, 152, 153, 187, 189, 192,
193, 194, 195
well-being, 148, 205
Western Blot IGG® kit, 57
Western lowland gorilla, 49, 144, 149
white-tailed deer, 10, 103
wildlife, 8, 12, 15, 21, 46, 64, 70, 94, 95, 123,
132, 135, 137, 139, 148, 180
wildlife health, 70
wildlife veterinarian, 180
wound treatment, 178

[X]
xylazine, 33, 34, 107

[Y]
yellow-eyed penguin, 21

[Z]
zebra, 126
zolazepam, 184
zoo animals, 96, 146, 195, 206
zoo leaders, 151, 152, 153
zoo veterinary community outreach, 154
zookeepers, 214
zoos, 43, 81, 122, 127, 142, 151, 179, 189, 190,
194, 195