TABLE OF CONTENTS
TABLE OF CONTENTS

Schedule of Presentations and Events 9

Abstracts 17

Session I  Human-wildlife Co-existence 65
Securing human-elephant coexistence
Simon Hedges, IUCN/SSC Asian Elephant Specialist Group

An update on the Asian Elephant Conservation Fund supported projects
Mini Nagendran, Asian Elephant Conservation Fund - U. S. Fish and Wildlife Service

The Elephants and Bees Project: Using bees as a natural deterrent for crop-raiding elephants
Lucy King, Save the Elephants

Session II  Illegal Trade 226
Ivory and rhino horn: Legal sale versus illegal poaching
W. R. Allen, The Paul Mellon Laboratory

Conserving African elephants through walking and talking over 2500 km in Kenya
Jim Nyamu, Elephant Neighbors Center

Section III  In situ Population Dynamics and Conservation 290
Focus on black rhino: Diceros bicornis bicornis population dynamics and a formula for successful conservation of the species 2002 - 2012
Susan P. Downie, David Shepherd Wildlife Foundation

Reproductive parameters in wild Asian elephants in Southern Sri Lanka estimated through individual-based longitudinal monitoring
Shermin De Silva, Colorado State University/Trunks & Leaves, Inc.

Determining potential environmental and social factors affecting the success of the black rhinoceros in Addo Elephant National Park, South Africa
Rachel Santymire, Davee Center for Epidemiology and Endocrinology Lincoln Park Zoo

White rhinoceros reproduction: Insights from the wild and semi-wild
Ron Swaisgood, San Diego Zoo Institute for Conservation Research

386
Session IV Reproduction I
Attempt to control estrus and ovulation in white rhinoceros using a synthetic progestagen and slow-release GnRH analogue
Annemieke van der Goot, University of Western Australia

Session V Veterinary Care
Manual restraint and chemical immobilization with xylazine/ketamine of wild and captive Sumatran elephants (*Elephas maximus sumatranus*) under field conditions
Christopher Stremme, Veterinary Society for Sumatran Wildlife Conservation

Urinary hormone concentrations and pharmacokinetics/pharmacodynamics of haloperidol in a female Indian rhinoceros (*Rhinoceros unicornis*)
Anri Benco, Cincinnati Zoo’s Center for Conservation and Research of Endangered Wildlife and Ohio State University

Current studies on molecular mechanisms of iron homeostasis in rhinoceroses
Rose Linzmeier, UCLA School of Medicine

Issues of elephant health care management in Myanmar Timber Enterprise (MTE), Myanmar
Zaw Min Oo, Ministry of Environmental Conservation and Forestry, Myanmar Timber Enterprise

Update from the Stakeholders Task Force for the Management and Research Priorities of Tuberculosis for Elephants in Human Care
Kay Backues, American Association of Zoo Veterinarians Representative to the Elephant Care Task Force and Tulsa Zoo

Testing for tuberculosis in elephants: what is the evidence?
David Miller, Stakeholders Task Force for the Management and Research Priorities of Tuberculosis for Elephants in Human Care

Point prevalence and incidence of *Mycobacterium tuberculosis* complex in captive elephants in the United States of America
Ramiro Isaza, University of Florida at Gainesville

Session VI Veterinary Care – Viruses
First evidence of EEHV infection in Sumatran elephants (*Elephas maximus sumatranus*) in Indonesia
Christopher Stremme, Veterinary Society for Sumatran Wildlife Conservation

Seven species of elephant endotheliotropic herpesviruses (EEHVs) form a novel mammalian subfamily, the Deltaherpesvirinae
Gary Hayward, The Johns Hopkins School of Medicine
Elephant herpesviruses EEHV2, EEHV3A, EEHV3B (a new subspecies), EEHV6, EEHV7A, EEHV7B (a new Subspecies) and EGHV1A, EGHV1B (a new species), EGHV2, EGHV4 found in tissue biopsies and saliva from African elephants in Kenya and America

Virginian Pearson, Princeton University

Elephant endotheliotropic herpesvirus (EEHV): where we are, where we are going

Lauren Howard, Houston Zoo

The lonely rhino: analyzing anthropomorphism toward solitary animals

Selenia Murillo, Chicago Zoological Society - Brookfield Zoo

Session VII  Ex situ Benefits and Support of In situ Conservation

The bigger picture – How captive elephant facilities benefit wild elephant populations

Sean Hensman, Elephants for Africa Forever

Contributions to science and conservation by elephant managers and captive elephants

Heidi Riddle, Riddle’s Elephant and Wildlife Sanctuary and Elephant Managers Association

Contributions of the Ringling Bros. Center for Elephant Conservation to wildlife management in Sri Lanka

Charles Santiapillai, Center for the Study of Asian Elephant at Rajarata University of Sri Lanka at Mihintale

Session VIII  In situ Conservation

Control of invasive arenga palm (Arenga obtusifolia) in habitat suitable for Javan rhino (Rhinoceros sondaicus), Ujung Kulon National Park, Indonesia

Sectionov Inov, International Rhino Foundation - Indonesia

The Role of standing sedation in mitigating human-elephant conflict in Sri Lanka

S. Wijeyamohan, Ringling Bros. Center for the Study of Asian Elephants at Rajarata University

Trend analysis of temporal and spatial patterns of human-elephant conflict in Nepal

Dinesh Neupane, Arkansas State University

Session IX  In situ Management of Wildlife and Habitat

The significance of pre-existing social bonds in translocated black rhinos

Natasha Anderson, Lowveld Rhino Trust

Boma adaptation and development of a scoring system for recently captured white rhinoceros (Ceratotherium simum) in South Africa

Michele Miller, Rare Species Conservancy Foundation

Contact calls of the Northern and Southern white rhinoceros: Source of information on individual identity and species of the caller?

Ivana Cinkova, Palacky University
Human - Elephant Conflict in the North West Wildlife Zone of Sri Lanka
*Pubudu Weerarathna, Species Conservation Center*

**Session X Reproduction II**

Oral imipramine and intravenous xylazine for pharmacologically-induced ex copula ejaculation in an African elephant (*Loxodonta africana*)
*Ray Ball, Tampa’s Lowry Park Zoo*

Pretreatment of Asian elephant (*Elephas maximus*) spermatozoa with cholesterol-loaded cyclodextrins and glycerol addition at 4 degrees C improves cryosurvival
*Wendy Kiso, The Ringling Bros. Center for Elephant Conservation*

**Session XI Ex situ Reproduction and Management**

Relationships among birth presentation, amniotic sac rupture and stillbirths in rhinoceroses
*Jane Kennedy, San Diego Zoo Global*

Thermoregulation in the African elephant and possible effects on fertility
*Ray Ball, Tampa’s Lowry Park Zoo*

Reproduction and population performance in the European captive population of Eastern black rhinoceroses (*Diceros bicornis michaeli*)
*Katie L. Edwards, Chester Zoo*

Improving the welfare of captive Asian elephants in Kerala, India
*T.P. Sethumadhaven, Kerala Veterinary and Animal Sciences University*

Relationship between management, adrenal activity and reproduction in a captive group of female Asian elephants (*Elephas maximus*)
*Jess Trotter, Chester Zoo*

Social and reproductive behavior of critically endangered Northern white rhinoceroses (*Ceratotherium cottonii*) in a zoological garden
*Ivana Cinkova, Palacky University*

**Ex situ Management**

Management of a breeding herd of African elephants (*Loxodonta africana*) on a predominately forage diet
*Ray Ball, Tampa’s Lowry Park Zoo*

Asian Elephant Support: A year in review and the importance of collaboration
*Linda Reifschneider, Asian Elephant Support*
Posters 1286

Symposium Participants 1296

Host Institution – International Conservation Center 1305

3rd Annual Management and Research Priorities of Tuberculosis for Elephants in Human Care Workshop Proceedings 1341
SCHEDULE OF PRESENTATIONS AND EVENTS
### Monday, 26 August 2013
10:00 – 18:00  Registration

18:00 – 20:00  Ice-breaker at the Comfort Inn

### Tuesday, 27 August 2013  All Presentations at the Comfort Inn

07:00 – 18:00  Registration

08:00 – 08:10  Welcome from the Pittsburgh Zoo & PPG Aquarium, International Elephant Foundation, and International Rhino Foundation

#### 08:10 – 10:10  Human-wildlife Co-existence

08:10 – 08:50  Securing human-elephant coexistence  
*Simon Hedges, IUCN/SSC Asian Elephant Specialist Group*

08:50 – 09:10  An update on the Asian Elephant Conservation Fund supported projects  
*Mini Nagendran, Asian Elephant Conservation Fund - U. S. Fish and Wildlife Service*

09:10 – 09:30  Rhinos: Are we at the tipping point?  
*Susie Ellis, International Rhino Foundation*

09:30 – 09:50  The Africa Asia Human Elephant Conflict Education & Resolution Project  
*Ravi Corea, Sri Lanka Wildlife Conservation Society*

09:50 - 10:10  The Elephants and Bees Project: Using bees as a natural deterrent for crop-raiding elephants  
*Lucy King, Save the Elephants*

10:10 – 10:30  Break

#### 10:30 – 12:10  Illegal Trade

10:30 – 10:50  Distinguishing between genuine and imitation rhinoceros horns and horn artifacts  
*Vanessa Blount, U. S. Fish and Wildlife Service Forensics Lab*

10:50 – 11:10  Assigning the provenance of African elephants using mitochondrial DNA  
*Alfred Roca, University of Illinois at Urbana-Champaign*

11:10 – 11:30  Ivory and rhino horn: Legal sale versus illegal poaching  
*W. R. Allen, The Paul Mellon Laboratory*

11:30 – 11:50  Is habitat or food plants responsible for rhino poaching in Kaziranga National Park, Assam, India?  
*Abhijit Rabha, Department of Environment and Forests, KAAC, Karbi Anglong, Assam and Bhrigu Prasad Saikia, Centre for Animal Ecology and Wildlife Biology, Gauhati University*

11:50 – 12:10  Conserving African elephants through walking and talking over 2500 km in Kenya  
*Jim Nyamu, Elephant Neighbors Center*

12:10 – 13:10  Lunch Buffet
<table>
<thead>
<tr>
<th>Time</th>
<th>Session III</th>
<th>Topic</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:10 – 13:50</td>
<td>In situ Population Dynamics and Conservation</td>
<td>Conservation genetics of greater one-horned rhinos in India – from counting numbers to determining conservation priorities</td>
<td>Udayan Borthakur, Aaranyak</td>
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<tr>
<td>13:50 – 14:10</td>
<td>Revealing cryptic forest elephant behavior through acoustics and thermal imaging</td>
<td>Peter Wrege, Cornell University</td>
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<td>14:10 – 14:30</td>
<td>Focus on black rhino: <em>Diceros bicornis bicornis</em> population dynamics and a formula for successful conservation of the species 2002 - 2012</td>
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<tr>
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<td>White rhinoceros reproduction: Insights from the wild and semi-wild</td>
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<th>Time</th>
<th>Break</th>
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<tbody>
<tr>
<td>15:30 – 15:50</td>
<td>Session IV</td>
<td>Reproduction I</td>
</tr>
<tr>
<td>15:50 – 16:50</td>
<td>Gestational pattern in elephants - their consequences for pregnancy and birth management</td>
<td>Thomas Hildebrandt, Leibniz Institute for Zoo and Wildlife Research</td>
</tr>
<tr>
<td>16:50 – 17:10</td>
<td>Relationship of salivary hormone concentrations to urinary hormone excretion profiles in the Indian rhinoceros (<em>Rhinoceros unicornis</em>)</td>
<td>Melissa Nau, Cincinnati Zoo’s Center for Conservation and Research of Endangered Wildlife and Ohio State University</td>
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<tr>
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<td>Attempt to control estrus and ovulation in white rhinoceros using a synthetic progestagen and slow-release GnRH analogue</td>
<td>Annemieke van der Goot, University of Western Australia</td>
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<tr>
<td>17:30 – 17:50</td>
<td>Correlation between serum progesterone levels and luteal blood flow in Asian elephants (<em>Elephas maximus</em>)</td>
<td>Stephan Botha, University of Guelph and African Lion Safari</td>
</tr>
<tr>
<td>17:50 – 18:10</td>
<td>Incidence of reproductive tract leiomyoma in Indian rhinoceroses</td>
<td>Robert Hermes, Leibniz Institute for Zoo and Wildlife Research</td>
</tr>
</tbody>
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<thead>
<tr>
<th>Time</th>
<th>Movie Night and Reception in the Ballroom</th>
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</tr>
</thead>
<tbody>
<tr>
<td>20:00</td>
<td>Battle for the Elephants</td>
<td>Battle for the Elephants</td>
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<td>John Heminway, Writer and Filmmaker</td>
<td>John Heminway, Writer and Filmmaker</td>
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</tbody>
</table>
Wednesday, 28 August 2013   All Presentations at the Comfort Inn

07:00 – 10:00   Registration

08:00 – 10:00   Session V  Veterinary Care
08:00 – 08:20   Manual restraint and chemical immobilization with xylazine/ketamine of wild and captive Sumatran elephants (*Elephas maximus sumatranus*) under field conditions
  Christopher Stremme, Veterinary Society for Sumatran Wildlife Conservation
08:20 – 08:40   Standing sedation in Sumatran elephants (*Elephas maximus sumatranus*) using Detomidine and Butorphanol
  Frank Göritz, Leibniz Institute for Zoo and Wildlife Research
08:40 – 09:00   Urinary hormone concentrations and pharmacokinetics/pharmacodynamics of haloperidol in a female Indian rhinoceros (*Rhinoceros unicornis*)
  Anri Benco, Cincinnati Zoo’s Center for Conservation and Research of Endangered Wildlife and Ohio State University
09:00 – 09:20   Current studies on molecular mechanisms of iron homeostasis in rhinoceroses
  Rose Linzmeier, UCLA School of Medicine
09:20 – 09:40   Issues of elephant health care management in Myanmar Timber Enterprise (MTE), Myanmar
  Zaw Min Oo, Ministry of Environmental Conservation and Forestry, Myanmar Timber Enterprise
09:40 –10:00 Update from the Stakeholders Task Force for the Management and Research Priorities of Tuberculosis for Elephants in Human Care
  Kay Backues, American Association of Zoo Veterinarians Representative to the Elephant Care Task Force and Tulsa Zoo
10:00 – 10:20 Testing for tuberculosis in elephants: what is the evidence?
  David Miller, Stakeholders Task Force for the Management and Research Priorities of Tuberculosis for Elephants in Human Care
10:20 – 10:40  Point prevalence and incidence of *Mycobacterium tuberculosis* complex in captive elephants in the United States of America
  Ramiro Isaza, University of Florida at Gainesville

10:40 – 11:00   Break

11:00 – 13:20   Session VI  Veterinary Care - Viruses
11:00 – 11:20   First evidence of EEHV infection in Sumatran elephants (*Elephas maximus sumatranus*) in Indonesia
  Christopher Stremme, Veterinary Society for Sumatran Wildlife Conservation
11:20 – 11:40   Clinical signs, diagnosis and treatment of the first case of EEHV3-B in an elephant
  Ellen Bronson, Maryland Zoo in Baltimore
11:40 – 12:00   Seven species of elephant endotheliotropic herpesviruses (EEHVs) form a novel mammalian subfamily, the Deltaherpesvirinae
  Gary Hayward, The Johns Hopkins School of Medicine
12:00 – 12:20   Elephant herpesviruses EEHV2, EEHV3A, EEHV3B (*a new subspecies*), EEHV6, EEHV7A, EEHV7B (*a new subspecies*) and EGHV1A, EGHV1B (*a new species*), EGHV2, EGHV4 found in tissue biopsies and saliva from African elephants in Kenya and America
  Virginia Pearson, Princeton University
12:20 – 12:40  Elephant endotheliotropic herpesvirus (EEHV): where we are, where we are going  
**Lauren Howard, Houston Zoo**

12:40 – 13:00  Veterinary treatment and management for papilloma viral infection of captive Sri Lankan elephant (*Elephas maximus maximus*) in Colombo Zoological Garden  
**Jagath Jayasekara, National Zoological Gardens, Sri Lanka**

13:00 – 13:20  The lonely rhino: analyzing anthropomorphism toward solitary animals  
**Selenia Murillo, Chicago Zoological Society - Brookfield Zoo**

13:30  **Buses to the Zoo**

14:00 – 18:00  Lunch and tour of the Pittsburgh Zoo & PPG Aquarium

14:00 – 16:00  Silent Auction for IEF/IRF in Elephant Family Room

16:00 – 18:00  **Elephant and Rhino Immobilization Workshop** – Must be pre-registered  
**Jeff Zuba, San Diego Zoo Global**

18:00 – 19:00  Poster Session and Cocktails at Water’s Edge

18:30 – 20:30  Silent Auction moves to the PPG Aquarium

19:00 – 20:30  Dinner at the PPG Aquarium

20:30 – 22:00  Buses return to the hotel

**Thursday, 29 August 2013  All Presentations at the International Conservation Center**

07:00 – 07:30  Registration and Continental Breakfast at the Comfort Inn

07:15 – 07:30  **Buses to ICC**

09:30 – 12:30  Session VII  **Ex situ Benefits and Support of In situ Conservation**

09:30 – 10:10  The bigger picture – How captive elephant facilities benefit wild elephant populations  
**Sean Hensman, Elephants for Africa Forever**

10:10 – 10:50  Contributions to science and conservation by elephant managers and captive elephants  
**Heidi Riddle, Riddle’s Elephant and Wildlife Sanctuary and Elephant Managers Association**

**Charles Santiapillai, Center for the Study of Asian Elephant at Rajarata University of Sri Lanka at Mihintale**

11:10 – 11:30  Reproductive assessment of Sumatran elephants (*Elephas maximus sumatranus*) in Elephant Conservation Centers (ECC) across Sumatra  
**Imke Lüders, GEolifes- Animal Fertility and Reproductive Research**

11:40 – 12:20  What elephants can teach us about preventing cancer  
**Joshua Schiffman, Huntsman Cancer Institute, University of Utah**

11:50 – 12:30  Rhino Protection Unit and support extended by local villages to protect the remaining Sumatran rhinos in Bukit Barisan Selatan National Park, Indonesia  
**Arief Rubianto, Rhino Foundation of Indonesia**
12:30 – 13:30  Lunch at the ICC

13:30 – 15:30  Session VIII  In situ Conservation
   13:30 – 14:10  Control of invasive arenga palm (*Arenga obtusifolia*) in habitat suitable for Javan rhino (*Rhinoceros sondaicus*), Ujung Kulon National Park, Indonesia
      *Sectionov Inov, International Rhino Foundation - Indonesia*
   14:10 – 14:30  The Role of standing sedation in mitigating human-elephant conflict in Sri Lanka
      *S. Wijeyamohan, Ringling Bros. Center for the Study of Asian Elephants at Rajarata University*
   14:30 – 14:50  Which future for human-elephant coexistence in the Boucle du Mouhoun region, Burkina Faso?
      *Julian Marchais, Des Eléphants & des Hommes*
   14:50 – 15:10  The conservationists’ dilemma: A need for pragmatism regarding captive breeding of exotic wildlife
      *Donald Paglia, UCLA School of Medicine*
   15:10 – 15:30  Trend analysis of temporal and spatial patterns of human-elephant conflict in Nepal
      *Dinesh Neupane, Arkansas State University*

15:30 – 20:00  Tour and Dinner at ICC

20:00  Buses return to hotel

Friday, 30 August 2013  All Presentations at the Comfort Inn

08:00 – 10:00  Session IX  In situ Management of Wildlife and Habitat
   08:00 – 08:40  The significance of pre-existing social bonds in translocated black rhinos
      *Natasha Anderson, Lowveld Rhino Trust*
   08:40 – 09:00  Boma adaptation and development of a scoring system for recently captured white rhinoceros (*Ceratotherium simum*) in South Africa
      *Michele Miller, Rare Species Conservancy Foundation*
   09:00 – 09:20  Contact calls of the Northern and Southern white rhinoceros: Source of information on individual identity and species of the caller?
      *Ivana Cinkova, Palacky University*
   09:20 – 09:40  The vanishing Asian elephant corridor in the Brahmaputra Valley, Assam: A threat to Asian elephant conservation
      *Bhrigu Prasad Saikia, Centre for Animal Ecology and Wildlife Biology, Gauhati University*
   09:40 – 10:00  Human - Elephant Conflict in the North West Wildlife Zone of Sri Lanka
      *Pubudu Weerarathna, Species Conservation Center*

10:00 – 10:20  Break
10:20 – 12:00  
**Session X  Reproduction II**

10:20 – 10:40  GnRH vaccination as a treatment for reproductive tract pathologies in female elephants (Contraception for post-reproductive cows: Why close the door after the cow is out?)
*Nancy Boedeker, Smithsonian's National Zoological Park*

10:40 – 11:00  Oral imipramine and intravenous xylazine for pharmacologically-induced ex copula ejaculation in an African elephant (*Loxodonta africana*)
*Ray Ball, Tampa's Lowry Park Zoo*

11:00 – 11:20  Suppression of testicular function by means of a GnRH vaccine in African elephant bulls
*Imke Lüders, GEOflies- Animal Fertility and Reproductive Research*

11:20 – 11:40  Pretreatment of Asian elephant (*Elephas maximus*) spermatozoa with cholesterol-loaded cyclodextrins and glycerol addition at 4 degrees C improves cryosurvival
*Wendy Kiso, The Ringling Bros. Center for Elephant Conservation*

11:40 – 12:00  Successful cryopreservation of Asian elephant (*Elephas maximus*) semen using simple low-tech techniques
*Danielle Arnold, University of Guelph and African Lion Safari*

**12:00 – 13:00**  
**Lunch Buffet**

13:00 – 15:00  **Session XI  Ex situ Reproduction and Management**

13:00 – 13:20  Relationships among birth presentation, amniotic sac rupture and stillbirths in rhinoceroses
*Jane Kennedy, San Diego Zoo Global*

13:20 – 13:40  Thermoregulation in the African elephant and possible effects on fertility
*Ray Ball, Tampa's Lowry Park Zoo*

13:40 – 14:00  Reproduction and population performance in the European captive population of Eastern black rhinoceroses (*Diceros bicornis michaeli*)
*Katie L. Edwards, Chester Zoo*

14:00 – 14:20  Improving the welfare of captive Asian elephants in Kerala, India
*T.P. Sethumadhaven, Kerala Veterinary and Animal Sciences University*

14:20 – 14:40  Relationship between management, adrenal activity and reproduction in a captive group of female Asian elephants (*Elephas maximus*)
*Jess Trotter, Chester Zoo*

14:40 – 15:00  Social and reproductive behavior of critically endangered Northern white rhinoceroses (*Ceratotherium cottoni*) in a zoological garden
*Ivana Cinkova, Palacky University*

**15:00 – 15:20**  
**Break**
15:20 – 17:30  Session XII  Ex situ Management

15:20 – 15:40  Comparison of visual body condition scoring systems in Asian elephants and validation by transcutaneous ultrasound
Kibby Treiber, Fort Worth Zoo

15:40 – 16:00  Body condition scoring index for female African elephants validated with ultrasound measurements of subcutaneous fat
Kari Morfeld, Smithsonian Conservation Biology Institute, National Zoological Park

16:00 – 16:20  The effect of fresh forages on the fat soluble vitamin and lipid profiles of greater one-horned rhinoceros (Rhinoceros unicornis)
Priya Bapodra, Columbus Zoo

16:20 – 16:40  Management of a breeding herd of African elephants (Loxodonta africana) on a predominately forage diet
Ray Ball, Tampa’s Lowry Park Zoo

16:40 – 17:00  Sex differences in captive elephant calf social interactions
Bob Dale, Butler University

17:00 – 17:20  Asian Elephant Support: A year in review and the importance of collaboration
April Yoder, Asian Elephant Support

17:20 – 17:30  Closing and Thank You
Deborah Olson, International Elephant Foundation

18:30  Buses leave for Pittsburgh Zoo

19:30  Movie Night and Reception at the Pittsburgh Zoo
War Elephants
David Hamlin, Wildlife Film Producer and Jared Lipworth, Executive Producer
National Geographic Television

20:45  Buses return to hotel

Saturday, 31 August 2013  Post Conference Tour to The Wilds

8:00  Bus leaves the Comfort Inn for The Wilds
10:30  Tour and lunch at The Wilds
15:00  Bus returns to the Comfort Inn
ABSTRACTS
Table of Contents

Session I:  Human - Wildlife Coexistence  ................................................................. 2-3
Session II:  Illegal Trade .................................................................................. 4-5
Session III:  *In situ* Population Dynamics and Conservation ...................... 5-8
Session IV:  Reproduction I .............................................................................. 8-10
Session V:  Veterinary Care ............................................................................. 10-14
Session VI:  Veterinary Care - Viruses ............................................................... 14-18
Session VII:  *Ex situ* Benefits and Support of *In situ* Conservation .............. 18-24
Session VIII:  *In situ* Conservation ................................................................. 24-27
Session IX:  *In situ* Management of Wildlife and Habitat .............................. 27-30
Session X:  Reproduction II .............................................................................. 30-34
Session XI:  *Ex situ* Reproduction and Management ...................................... 34-36
Session XII:  *Ex situ* Management ................................................................. 37-40
Posters ........................................................................................................... 40-44
Abstracts
Session I: Human - Wildlife Co-existence

Securing Human–Elephant Coexistence
Simon Hedges, IUCN/SSC Asian Elephant Specialist Group

Elephants are wonderful creatures with complex social lives, play a dominant role in the ecosystems in which they live, and act as flagships for the conservation of biodiversity wherever they occur. Elephants are also of great economic significance: for example, they do great damage to crops, especially large-scale plantations, but they are also sources of income for many people through wildlife-viewing-based tourism, especially in Africa. In addition, elephants are of tremendous cultural and religious significance across Asia. It has been said that, “One can start from Ganesha and work from there in an unbroken line to almost any aspect of Indian culture.” Similar observations could be made about the role of elephants in the cultures of many other countries in Asia. Unfortunately, elephants are threatened by habitat loss, fragmentation, and degradation; illegal killing for ivory and other products or in retaliation for human–elephant conflict; and, in some regions at least, a loss of genetic viability resulting from small population size and isolation. Moreover, a lack of good-quality data on population size, distribution, and even basic biology hampers effective elephant conservation in many areas. Furthermore, in too many range States a lack of capacity in wildlife departments also handicaps effective conservation. But it does not have to be like this. There is still time to turn this situation around; there are still enough elephants and enough wild places for the species to thrive again.

To address the various threats, it is necessary for the international community to work with range State governments to secure large new protected areas for elephants and other wildlife; to quantify and reduce illegal killing through improved law enforcement measures; to assess and field-test a range of both traditional and novel approaches to reducing human–elephant conflict, promoting those methods shown to be effective; and to evaluate, and promote new techniques for elephant monitoring. However, while actions at the site and landscape level are necessary to save elephants they are not sufficient. It is also necessary to act outside of sites and landscapes at the provincial, national, and global scales. Many important threats to elephants including trade in elephants and elephant parts (especially ivory) and the impacts of the large scale extractive industries and of infrastructure developments cannot be addressed at the site or even landscape level. It is necessary, therefore, to identify and disseminate best practices and to work to directly influence policy in those arenas particularly important to elephant conservation.

Fortunately, there are signs that evidence-based approaches to elephant conservation are having an increasing, if overdue, impact in Asia and Africa and that the global community is once again acting in the face of the new African elephant poaching crisis, and so we may yet secure human–elephant co-existence across large parts of their range. These welcome developments as well as the remaining very significant challenges are reviewed.

An Update on the Asian Elephant Conservation Fund Supported Projects
Mini Nagendran, Asian Elephant Conservation Fund - U.S Fish and Wildlife Service

The Asian Elephant Conservation Fund of the US Fish and Wildlife Service has provided support for field conservation projects in the 13 Asian range countries since 1999. The primary threat to Asian elephants is continued habitat loss leading to increasing human-elephant conflict. The presentation will discuss success stories, a range-wide estimate of wild Asian elephant populations, threats and challenges.

Rhinos: Are We at the Tipping Point?
Susie Ellis, Ph.D., International Rhino Foundation

Rhinos have walked the Earth for more than 50 million years, but today, all but one species faces extinction, possibly within our lifetimes. Organized poaching in Africa and India is taking place at nearly unprecedented rates, and Indonesian rhinos face a host of existing and emerging threats. But the news is not all bad. This presentation provides an update on the current situation facing the five rhino species and outlines some of the most urgent rhino conservation and research needs — requiring all our collaborative efforts to prevent extinction.

The Africa Asia Human Elephant Conflict Education & Resolution Project
Ravi Corea, Sri Lanka Wildlife Conservation Society

From time immemorial people have been fascinated and been in awe of elephants as well as terrified of them. In Asia this fascination had led to the development of one of the most unique relationships that had ever evolved between a wild animal and a human. In Africa most unfortunately the fascination for the elephant is mostly to do with its ivory rather than for its’ enigmatic and wondrous nature. Today irrespective what their relationships with people had been historically, both species of elephants are in critical trouble. The Africa Asia Human Elephant Conflict Education & Resolution Project will be a pioneering effort—for the first time African and Asian conservationists will partner to develop effective measures to address the decline of both species due to intense human-elephant conflicts (HEC).
Poaching for ivory is the biggest crisis for the African elephant, yet HEC is a covert and equally lethal process that decimates elephants over the long term. In Asia HEC contributes the most to elephant mortality yet poaching still occurs and is a threat with the potential to increase influenced by events happening in Africa and due to the increasing demand for ivory. Basically elephants are being mowed down from both ends and at this rate of killing it is doubtful whether both species will survive to see the dawn of another century. The Africa Asia Human Elephant Conflict Education & Resolution Project is strategically important due to the following reasons:

1. The current crisis the African elephant is facing due to poaching and HEC.
2. The increasing HEC in Asia due to exponential development and population growth.
3. The lack of collaboration between Asian and African counterparts.
4. The project is about protecting the planet’s natural resources.
5. Killing of elephants is also a national security issue, a public health issue and an economic security issue since dead elephants will affect the tourism dollars of Africa and Asia in the long term.

The Sri Lanka Wildlife Conservation Society has been addressing HEC mitigation for the past 17 years and the society’s Saving Elephants by Helping People Project received a UNDP Equator Prize in 2008. The SLWCS is keen to use its experience as well as learn about HEC resolution in Southern Africa. There must be HEC situations in Africa and Asia that are similar but are looked upon and addressed differently due to widely differing social, cultural, economic, management, and political perspectives. Therefore combining our collective experience and knowledge would provide inspiration to develop several innovative measures to establish the world’s first such joint effort to benefit both African and Asian elephants.

The project brings together five conservation groups: the Sri Lanka Wildlife Conservation Society, Integrated Rural Development and Nature Conservation in Namibia, the Elephant Pepper Development Trust in Zimbabwe and the Okavango Elephants & People Research Project in Botswana in a unique partnership where we will work together to develop new models to mitigate human elephant conflicts. The project will create a platform to assess and discuss ideas in the field. One of the important outcomes would be the publication of a human elephant conflict field manual. This would be an illustrated guide drawn in the same way airline safety cards are: A simple easy to understand drawings with minimum text. The Field Manual will provide graphically illustrated information about HEC and how to address it. The Field Manual will be designed so that it can be easily adapted to regional conditions and languages. The guide will be translated into regional languages, and laminated to withstand the rigors of remote wilderness application. These field manuals will be distributed to areas most affected by human elephant conflict, and provide practical advice on how to lessen the frequency of HEC incidents such as safeguarding crops and property and protecting human life with minimum harm to elephants.

The seed money for this seminal project was provided by the International Elephant Foundation.

The Elephants and Bees Project: Using Bees as a Natural Deterrent for Crop-Raiding Elephants

Lucy E. King 1,2*, Iain Douglas-Hamilton1,2 and Fritz Vollrath1,2

1 Animal Behaviour Research Group, University of Oxford
2 Save the Elephants

Increasing elephant populations in Kenya since 1989 have been widely praised as a conservation success story. However, where elephants and agricultural land overlap, incidents of human–elephant conflict are on the increase. Wildlife managers and farmers are now trying different farm-based deterrents to keep elephants out of crops. Here, we present data on the effectiveness of a novel beehive fence deployed in a Turkana community of 62 communally run farms in Kenya. Specifically, 1700-m of beehive fences semi-surrounded the outer boundaries of seventeen farms, and we compared elephant farm invasion events with these and to seventeen neighbouring farms whose boundaries were “protected” only by thorn bush barriers. We present data from 45 farm invasions, or attempted invasions, recorded over 2 years. Thirteen groups of elephants approached the beehive fences and turned away. Of the 32 successful farm invasions, only one bull elephant broke through the beehive fences. These results demonstrate that beehive fences are more effective than thorn bush barriers at deterring elephants and may have a role to play in alleviating farmer–elephant conflict. Additionally, the harvesting of 106 kg of honey during the trial period suggests that beehive fences may also improve crop production and enhance rural livelihoods through honey sales.
Session II: Illegal Trade

Distinguishing Between Genuine and Imitation Rhinoceros Horns and Horn Artifacts
V.M. Blount, B.C. Yates, and E.O. Espinoza, National Fish and Wildlife Forensics Laboratory

The National Fish and Wildlife Forensics Laboratory assists U.S. Fish and Wildlife Service agents investigate criminal operations involved in illegally obtained rhino horns for sale in the global wildlife trade. This contraband includes raw horns, fragments of horns, carvings, and artifacts. Successful prosecution depends on correct taxonomic identification of each evidence item. Forensic protocols include morphological identification, genetic assessment, and keratin characterization via Fourier Transform Infrared Spectroscopy. Items are examined initially for probable cause using both covert and in situ photographic assessment documenting morphological details, and after seizure, physical and instrumental analyses are performed. Results of these analyses reveal trafficking of both black and white rhino horns, as well as an assortment of deliberate fabrications of rhino horn fakes.

Assigning the Provenance of African Elephants Using Mitochondrial DNA
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African elephant mitochondrial (mt) DNA follows a distinctive evolutionary trajectory. As females do not migrate between elephant herds, mtDNA exhibits low geographic dispersal. We therefore examined the effectiveness of mtDNA for assigning the provenance of African elephants (or their ivory). For 653 savanna and forest elephants from 22 localities in 13 countries, 4258 bp of mtDNA was sequenced. We detected eight mtDNA subclades, of which seven had regionally restricted distributions. Among 108 unique haplotypes identified, 72% were found at only one locality and 84% were country specific, while 44% of individuals carried a haplotype detected only at their sampling locality. We combined 316 bp of our control region sequences with those generated by previous trans-national surveys of African elephants. Among 101 unique control region haplotypes detected in African elephants across 81 locations in 22 countries, 62% were present in only a single country. Applying our mtDNA results to a previous microsatellite-based assignment study would improve estimates of the provenance of elephants in 115 of 122 mis-assigned cases. Nuclear partitioning followed species boundaries and not mtDNA subclade boundaries. For taxa such as elephants in which nuclear and mtDNA markers differ in phylogeography, combining the two markers can triangulate the origins of confiscated wildlife products. Published in Evolutionary Applications 6(2):253-265, 2013. Supported by USFWS African Elephant Conservation Fund Grant Number 0554-96200-0-G051.

Ivory and Rhino Horn: Legal Sale Versus Illegal Poaching
W.R. Allen
The Paul Mellon Laboratory

The unsustainably high levels of poaching of elephant and rhino blighting many African countries at present are driven by deeply rooted traditional pressures in China and other Asian countries for intricately carved ivory ornaments and an imagined pharmacological panacea. The present CITES listing of elephant and rhino in Appendix 1 effectively bans all international transport of, and trade in, ivory and rhino horn. Clearly it is failing badly and it fuels the existence of the ruthless middleman racketeer element which sustains the poaching industries at both the supply and user ends of the chain.

This paper reviews current methods and levels of poaching and illegal sale of ivory and rhino horn and it argues for a radical new approach to address the problem. First, dialogue with authoritative representatives of both the ivory carving and traditional medicine industries in China/Asia to determine realistic figures for present demand for both products and likely future requirements were they to become legally available at reasonable prices. Second, dialogue with high ranking CITES representatives to determine the theoretical and administrative difficulties of instigating an experimental, say 5 year, relaxation of the existing blanket ban on the sale and transport of ivory and rhino horn. Third, dialogue with senior and knowledgeable representatives of National Game Parks, Hunting Conservancies and Governments of the major sub-Saharan African countries affected by the current poaching storm, to list their present stocks of ivory and rhino horn, estimate the numbers of live “useable” elephant and rhinos surviving in their country and calculate how much of both products they might be able to sell sustainably. Fourth, discuss with appropriate authorities and governments the establishment of a pan-African tightly controlled and fully transparent marketing organization to regulate the legal sale of ivory and rhino horn at reasonable prices to reputable dealers in China and elsewhere, with equable return of income to the supplying countries and to their farmers and/or indigenous peoples who have produced or protected each species to enable such humane and sustainable sale of their products.

Can sufficient rhino be farmed commercially for their horn, along similar lines to the production of Red deer velvet in New Zealand? Could enough ivory be gathered from existing stocks, natural deaths and population control culling to meet reasonable demands of the user markets in Asia. Most important, could the proposed marketing organization be established and run well enough to knock the bottom out of and sink the illegal poaching industry?
The Indian rhino has been listed as Vulnerable in the IUCN Red Data Book (2012), CITES Appendix-I species and Schedule-I species of the Indian Wildlife (Protection) Act, 1972 to ensure the conservation of this mega-herbivore. The Kaziranga National Park, Assam, has the highest population of Indian rhino, which are heavily threatened by poaching pressure.

There have been 115 Indian rhinos killed during the last 10 years in Kaziranga National Park (KNP), Assam, through the year 2012. The records suggest that poaching increases as the number of Indian rhinos increase within Kaziranga National Park. It has also been noted that most Indian rhinos are killed in the habitats along the boundary line of the KNP. Hence, the present study was conducted with the objective to evaluate the habitat utilization and feeding patterns of stray Indian rhinos in KNP during the year 2011-2012.

The representative habitats present along the boundary line of KNP have been identified and GPS data were collected for the classification of the habitat using ERDAS and ArcGIS 10 software. The habitat utilization pattern data were collected by using the ‘dawn to dusk’ scan animal and ad libitum sampling methods for each of the habitats utilized by Indian rhino. The feeding pattern study wouas also conducted by ‘dawn to dusk’ scan animal and ad libitum sampling methods; data on the food plant species utilized during the time they strayed outside the KNP were also collected by direct observation.

It has been found that, the Asian elephant utilizes the grassland habitat in Kaziranga National Park but occupies the woodland habitat outside of park boundaries. In contrast, stray rhinos mostly feed in the grassland, utilizing habitats in a mosaic manner outside the KNP boundary. During the present study it was noted that the distribution of the stray rhinoceros depends on habitat and food availability. There was a strong association observed between the Indian rhino poaching sites and the presence of grassland and wetland in the surrounding areas. Habitat and food shortages lead to increased straying behavior of the Indian rhino in Kaziranga National Park, which negatively impacts Indian rhino conservation because they are more likely to get killed while visiting these grasslands outside the protected areas. However, annual floods also force the Indian rhino to come out of the protected boundary of KNP making it vulnerable to poaching.

Hence, for the conservation of the Indian rhino outside the boundary of the Kaziranga National Park, the grassland and wetland habitats need to be protected. The grassland should be managed properly to ensure food availability for the Indian rhino. The Indian rhino does not have any political or legal protection when it strays outside the KNP boundary to fulfill its ecological needs; therefore protection should expand to include areas beyond the KNP boundaries during times of ecological crisis like annual floods, food shortages, etc. when Indian rhinos are likely to stray outside the Kaziranga National Park.

Conserving African Elephants Through Walking and Talking Over 2500-km in Kenya:

Jim Nyamu

Kenya continues losing at least 7-10 elephants per week. In the year 2012, Kenya lost 450 elephants with most parts of the protected areas losing more than 3 elephants per day. At least 145 elephants have been poached this year and if the status remains by the end of the year, more than 300 elephants will be killed. In 1979 Kenya had 167,000 elephants that were drastically reduced to 16,000 by 1989. The population has gradually been increasing to 30,000 currently (2013) with threatening poaching and habitats degradation all over the country.

I have walked over 2,500-km across Kenyan coast, Rift Valley, Northern Kenya, Eastern and Central Kenya using “Ivory belongs to elephant” slogan. During the 64 days walk I had three objectives (1) Raising awareness on elephant poaching (2) Raising awareness on elephant values in regards to economic and ecological values and (3) Engaging local communities on how to mitigate human-elephant conflict. It’s apparently clear that 78% (n-2030) people do not want to conserve our precious elephants despite the fact that 80 % of wildlife in Kenya is found outside the protected areas.

The walk brought together over 50 national and international conservation organisation who pledged to support the upcoming walk in East Africa in October 2013 as we establish community based conservation program.

Session III: In situ Population Dynamics and Conservation

Conservation Genetics of Greater One-horned Rhinos in India – from Counting Numbers to Determining Conservation Priorities

Udayan Borthakur, Aaranyak
Pranjali Kumar Das and Bibhab Kumar Talukdar, Aaranyak

Being a major flagship species in its range distribution and emerging from a catastrophic population decline in the past, the Greater One-horned Rhino (Rhinoceros unicornis) population management and conservation requires a multi-disciplinary effort, including the use of molecular tools for genetic monitoring of natural populations. In India, seven protected areas harbour more than 70% of the extant global population of this species. The contemporary populations of rhino are distributed across a landscape of fragmented habitat patches of protected areas, with restrictions to the natural movement and gene flow among them due to various land use patterns. This demanded an investigation on whether this distribution pattern facilitates any demographic and
genetic exchange of rhinos among the protected areas in India and whether any genetic management of these populations are required in order to ensure their future survival.

Keeping this in view, we have initiated genetic population monitoring of rhinos in 5 different protected areas of India, viz. Kaziranga National Park, Orang National Park, Pobitora Wildlife Sanctuary of Assam and Gorumara National Park and Jaldapara National Park of West Bengal, with 98% of the total rhinos present in the country. Microsatellite markers were optimized to identify individual rhinos and Y-chromosome linked markers to identify gender from noninvasively collected dung samples, along with optimization of sampling techniques to meet the requirements of genetic studies. Using these techniques, we have successfully carried out genetic census of rhinos in Gorumara, while using the same techniques to carry out a wider investigation of genetic population structuring and gene flow among the protected areas.

The result of our genetic census of rhinos in Gorumara was in concordance with the hear-count census carried out by the West Bengal Forest Department, and we have further confirmed that the population has a skewed sex ratio and a comparatively low level of genetic diversity. Genetic monitoring based on dung DNA analysis revealed direct evidence of demographic exchange of rhinos among the protected areas of Assam. In overall, genetic monitoring of rhinos revealed moderate to high levels of genetic diversity spreading across the rhino bearing protected areas, with a high degree of structuring observed. We are of the opinion that the techniques of genetic monitoring adopted by us, with further developments on a robust sampling strategy, can be used for estimating population size of rhinos in all the protected areas in future. These genetic techniques may further be useful in forensic investigation of rhino poaching cases, which is on a peak in Assam for past few months. Further, our analysis of spatial distribution of genetic diversity of rhinos in India shows the need of restocking the West Bengal population, in view of the lack of natural connectivity with other source populations.

Revealing Cryptic Forest Elephant Behavior Through Acoustics and Thermal Imaging

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1Wildlife Conservation Society; 2Cornell University

A sufficient understanding of the ecology and behavior of forest elephants (Loxodonta cyclotis), even for developing basic conservation strategies, has been difficult to achieve. These elephants spend the majority of their time in small, dispersed groups, or solitarily within rainforest habitats where direct observation is severely limited and dangerous. Although forest clearings often attract large numbers of elephants, observation at such sites has been limited by a number of factors, not the least of which is that elephants prefer to visit these clearings at night. Autonomous acoustic monitoring has proven valuable in both forest settings and at forest clearings for continuous tracking of relative abundance and to characterize patterns of both diel and seasonal activity. The addition of thermal imaging provides a means to probe nocturnal behavior and to interpret acoustic patterns for use in distributed monitoring situations.

This paper draws on seven years of acoustic monitoring to quantify patterns of forest elephant behavior that in some cases were suspected and in other cases unknown. In the forest, where these elephants spend close to 95% of their time budget, activity is nearly equally divided between daytime and nighttime periods, with significant but modest seasonal differences. However, activity in forest clearings is overwhelmingly nocturnal—assumed to be a response to the increased risk of being hunted in such sites. The proportion of all activity occurring at night, and changes in this proportion, could be valuable indicators of hunting pressure. Acoustic monitoring provides a metric of activity that is unbiased with respect to time of day, or phase of the moon, and should be implemented with or without observation teams at specific clearings. Change in the mix of acoustic signals produced by elephants in clearings at night suggests that different behaviors may be involved or perhaps a change in the mix of sex and age classes entering the clearing. A recent study that paired acoustic recording with thermal imaging confirmed that the number of elephants simultaneously in the clearing increased dramatically after dark, but that this increase was due disproportionally to female-calf groups. The number of adult males increased only slightly. Sexual behavior was an order of magnitude more frequent at night than during the day and was correlated with the previously detected change in vocalization types at night across multiple sites, providing the first indication that clearings may indeed function as mating hotspots.

Focus on Black Rhino (Diceros bicornis bicornis) Population Dynamics and a Formula for Successful Conservation of the Species 2002 - 2012

S.P. Downie; A. Mavrandonis
David Shepherd Wildlife Foundation

In 1989 South Africa had less than 20 Diceros bicornis bicornis. This project alone has helped quadruple that number. The Biodiversity Management Plan for the Black Rhinoceros in South Africa 2011 – 2020, (BMP) (Government Gazette, 25th January 2013), calls for 260 by 2020, and a longer term goal of 500.

This project, registered with South African National Parks, supports a number of strategic objectives of rhino monitoring and management by studying separate sub-populations in four smaller parks within their historical range. The accumulated data assesses and measures performance towards the BMP goals.

Although data is available for over 21 years, and the first founder population was introduced to one study area in 1999, regular ground monitoring commenced in 2002, and numbers have increased from 5 to 72 rhinos. All are individually identifiable. The objectives were to monitor developmental stages, study all performance indicators, assess dispersal into new habitats, determine success/failure of translocation strategies, study behaviour and develop genealogy.

The experience with Shibula, returned to southern Africa from Lisbon Zoo in 1991, has been an inspiration, was invaluable, and is comprehensively documented.
Determining Potential Environmental and Social Factors Affecting the Success of the Black Rhinoceros in Addo Elephant National Park, South Africa
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1Lincoln Park Zoo, 2Addo Elephant National Park, 3Western Kentucky University, 4George Mason University

Factors, including intra- and interspecies conflicts, may make it more difficult to increase population numbers of the critically endangered black rhinoceros (rhino). Using non-invasive methods to study this elusive species can assist with determining factors that limit its success in the wild. Our goal was to determine the health and success of a population of southwestern subspecies of black rhinos (Diceros bicornis bicornis) in Addo Elephant National Park (AENP). Camera traps were placed on rhino middens to identify individuals and facilitate the collection of fecal samples to measure adrenocortical activity (via fecal glucocorticoid metabolites, FGM). Four camera traps were set up in two park sections, Addo’s main campus (Addo; n=2 camera sites) and Nyathi (n=2 camera sites), which were separated by a highway and differ with respect to numbers of competitors, predators and tourists, which were higher in Addo than Nyathi. The photos and feces were used to investigate the relationships among season (wet vs. dry), number and sex of the rhinos that came to each site (n=4) and FGM concentrations. Additionally, GIS data from each site were used to determine the influence of landscape cover (xeric vs. mesic) and type of roads (staff vs. concession) on FGM concentrations. Fresh fecal samples (n=167) were collected, processed and sent to the United States for FGM analysis. Results demonstrate that individuals in Addo had higher (P<0.001) FGM concentrations compared to Nyathi rhinos. Specifically, one site in Nyathi had the lowest (P<0.001) FGM compared to the other three sites; however, these FGM values were not influenced by the number of different individual visitors to the sites, which ranged from 4 to 14 rhinos; nor by sex (ratio of females to males ranged from 0.33 to 3.0). In Addo, GIS data and type of roads did not influence (P>0.05) FGM values. However, neither of these factors differed among the camera trap areas in Nyathi (all were mesic and concession roads only); so comparisons could not be made. Irrespective of section, FGMs were higher (P<0.05) in wet versus dry season. Yet, the season did not seem to influence the number of rhinos at each site even the two sites with waterholes. In conclusion, our results may be limited by the camera trap placement on middens because the rhinos may be avoiding the highly disturbed areas (near tourists and heavily used roads) and other environmental pressures due to competitors and/or predators. However, overall, rhinos residing in Addo, the more disturbed section of AENP, did have higher adrenocortical activity suggesting interspecies conflicts may be negatively impacting the health of the population. Results can be used to assist AENP managers with improving the success of their rhino population.

Reproductive Parameters in Wild Asian Elephants in Southern Sri Lanka Estimated Through Individual-based Longitudinal Monitoring
Shermin de Silva1,2,*, C. Elizabeth Webber3, U. Sameera Weerathunga2, J.S. Tharanga2, T.V. Kumara2, Devaka K. Weerakoon4, George Wittemyer1
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High-resolution demographic data on wild Asian elephants has been difficult to collect due to the habitat characteristics of much of the species’ remaining range. Such data, however, is critical for understanding and modeling population processes in this endangered species. I present data from six years of an ongoing study of Asian elephants (Elephas maximus) in Udawalawe National Park, Sri Lanka. This relatively undisturbed population is individually monitored, providing cohort-based information on survival and fecundity. Reproduction was seasonal, such that most births occurred during the long inter-monsoon dry season and peaked in May. The average age at first reproduction was 11.4 years with an average inter-birth interval of 3.9 years. Birth sex ratios did not deviate significantly from parity. Fecundity was relatively stable throughout the reproductive life of an individual (ages 11-60), averaging 0.15 female offspring per individual per year. Mortalities and injuries based on carcasses and disappearances showed that males were significantly more likely than females to be killed or injured through anthropogenic activity. Overall, however, most observed injuries did not appear to be fatal. This population exhibits higher fecundity and density relative to other Asian elephant populations including those in captivity, possibly enhanced by present range constriction. Understanding the factors responsible for these demographic dynamics can shed insight on the future needs of this elephant population, with probable parallels to other populations in similar settings.

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Due primarily to poor reproduction among females born in captivity (F1 generation) the southern white rhinoceros (Ceratotherium simum simum) captive population is not self-sustaining. We devised a detailed in-person questionnaire to evaluate histories of individuals reared in varying conditions in small South African game reserves that lie on a continuum between wild and captive, with the goal of determining how social environment, reserve size, and husbandry factors (during development and as adults) influence reproduction. We obtained interviews from 96 properties. For the individual-female data, 96% of F1’s and 92% of F2’s reproduced successfully. For population-level data, 92% of the respondents indicated that their F1’s were reproducing. We also examined the reproductive rate of females and inter-birthing intervals, but found differences between F1 and F0 females did not approach significance. These data are markedly different from those in captive populations.

We also analyzed the effects of 9 independent variables that we hypothesized may influence rhino reproduction: (1) supplemental feeding intensity, (2) supplemental feeding frequency, (3) male social environment, (4) female social environment, (5) overall social density, (6) male social density, (7) female social density, (8) rainfall, and (9) property size. Analyses show that only two of these variables had significant effects: social density and supplemental feeding. Overall social density and female density had a significant effect, with female reproductive rates increasing with increasing density. This finding runs counter to the hypothesis that suppression by older females is reducing reproduction in F1 females in zoo environments; if dominance-mediated aggression and suppression were negatively impacting these semi-wild populations in South Africa, the opposite effect is predicted. Increased supplemental food was associated with higher reproduction. None of the independent variables had a differential effect on reproductive success between the generations (i.e., statistical interactions not significant), indicating that factors affecting reproduction affect both F1 and F0 females in the same way.

To evaluate possible developmental effects of the rearing environment, we examined the same 9 factors during the first 5 years of life. Our results mirror the adult statistics, with a positive relationship between rate of reproduction and social density measures, in contradiction to what would be expected if high densities during development negatively affected F1 reproductive success.

Some have hypothesized that zoos should keep more than one male to encourage reproduction in F1 females, but several populations in our study contained only a single male and had successful F1 reproduction. Thus, male-male competition and female mate choice are not prerequisites for reproduction, as has been suggested, and we should probably look elsewhere for better explanations.
occurring 2-5 days prior birth. Besides the analytical approach, the upcoming birth can also be predicted by daily transrectal ultrasound assessments. The frequent perinatal examinations requires, conditioning of the pregnant cow including rectal palpation and to foreign people as well as technical equipment of at least 6 months prior to the calculated birth date. The ultrasound examinations can be performed in direct and protected contact settings. The presentation will demonstrate a variety of data collected during routine ultrasound examinations in pregnant wild and captive elephants combined with pregnancy associated endocrine profiles and the corresponding gestational disorders observed at specific pregnancy stages.

Relationship of Salivary Hormone Concentrations to Urinary Hormone Excretion Profiles in the Indian Rhinoceros (Rhinoceros unicornis)
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Improperly timed introductions for mating can lead to severe aggression between male and female Indian rhinos. To minimize this risk, pairings or artificial insemination (AI) must be timed to ovulation as accurately as possible. Currently, urinary concentrations of estrogen conjugates (EC) and progesterone metabolites (PdG) are used to monitor ovarian function and time breeding or AI. Collecting clean urine samples consistently can be a major challenge with some rhinos, and has limited the number of individuals for which hormone monitoring can be used. Reproductive hormones can also be detected in saliva, and have been previously measured in a female Indian rhino. However, before saliva can be used as the sole biological sample for monitoring ovarian function in this species, it must be proven to be reliable, accurate and reflective of urinary hormone profiles.

Matched urine and saliva samples were collected from two multiparous female Indian rhinos (#137, #189) and analyzed using enzyme immunoassay (EIA) techniques. Samples were obtained during morning hours and frozen (-20°C) until analysis. Urinary EC (R522), PdG (R13904) and cortisol (R4866) concentrations were indexed by creatinine. Saliva samples were centrifuged (1500g) and the supernatant analyzed for testosterone (R156/7) and cortisol. A portion of salivary supernatant was ether extracted, concentrated and analyzed for progesterone (P4; Salimetrics, State College, PA). Three estrous cycles were monitored per female. Female #137 was paired for natural breeding during one estrous cycle, and female #189 had a non-anesthetic AI performed on each cycle. An ovulation inducing agent (Sucromate™; 2.1cc IM) was administered during two estrous cycles in female #189. Transrectal ultrasonography was performed (#189) to monitor follicular growth and verify ovulation. Ultrasonography was also employed (#137, #189) 21 days post-breeding/AI to diagnosis pregnancy. Urine and salivary steroid results were aligned with day 0 corresponding to day of behavioral estrus or day of sucromate injection. Pearson product moment correlation coefficients were calculated between urinary and salivary hormone concentrations.

Matched values of urinary EC and salivary testosterone were correlated throughout the estrous cycle (r=0.815; P<0.05). Profiles of urinary PdG and salivary P4 were also highly correlated (r=0.804; P<0.05). Despite the fact pooled saliva produced a displacement curve parallel to the standard curve for cortisol (r=0.99; neat to 1:8), salivary cortisol concentrations did not correlate (r=0.058; P=0.698) to urinary concentrations of cortisol. Urinary cortisol has been validated in this species using the R4866 antibody, but it appears additional research is needed to produce reliable salivary cortisol results. As a by-product of this study, we determined a cyclic variation in urinary cortisol concentrations occurs during the Indian rhino estrous cycle. Concentrations of urinary cortisol peaked on day 0 (45.14 ± 7.96 ng/mg Crt). Urinary cortisol and EC were positively correlated (r=0.681; P<0.05) throughout the estrous cycle, while urinary cortisol and PdG were negatively correlated (r=0.398; P<0.05). Although successful ovulation was documented in female #189, only female #137 became pregnant. Results from this study demonstrate that Indian rhino saliva samples yield temporal patterns of testosterone and P4 profiles consistent with urinary EC and PdG.

Acknowledgment: This study was supported by the P&G Wildlife Conservation Scholarship, Proctor & Gamble Pet Care, Cincinnati, OH.

Attempt to Control Estrus and Ovulation in White Rhinoceros Using a Synthetic Progestagen and Slow-release GnRH Analogue
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Breeding in captivity may play an important role in the survival of the conservation-dependent white rhinoceros (Ceratotherium simum). However, the overall reproductive rate in captive is too low, and the captive population is not self-sustaining. One related factor is the inexplicable observation at many institutions of an aberrant cycling pattern in females. In order to increase breeding success in captivity, it is important to determine the direct causes of estrous cycle irregularity and also to develop an estrus and ovulation synchronization protocol that allows timed natural breeding and assisted reproduction. Several studies have involved an attempt to induce estrus in the white rhinoceros, but ovulation using those protocols was inconsistent from female to female. This study utilized a progestagen treatment followed by a slow-release GnRH analogue to synchronize estrus and ovulation among 2 southern white rhinoceros. Oral synthetic progestagen (altrenogest, 0.022mg/kg/d) was administered for 21 days after a random start, followed by a single injection of a synthetic slow-release GnRH analogue (deslorelin acetate, 2.5 µg/kg) 9.5 days after discontinuing progestagen treatment. Treatment success was determined using enzyme immunoassay of daily fecal samples and behavioral observations. During the protocol, females were successfully introduced to a bull. Although no mating or estrus behavior was detected during or after treatment, fecal hormone analysis did show synchronized luteal activity in the females. Interestingly, this synchronization did not appear to be the result of the progestagen and GnRH protocol, and other causative factors should be considered.
Asian elephants (*Elephas maximus*) are one of the most endangered species of animals in the world with less than 30,000 remaining in the wild. An aging captive population along with difficulties in captive breeding has created a need for a better understanding of the Asian elephant’s unique reproductive system. Elephants are unique in the fact that they are mono-oestrous animals yet present multiple corpus lutea both during regular cycling and pregnancy. They also present two luteinizing hormone peaks a feature only found in elephants. Their main excreted progestagen is not progestrone as in most other mammals but it’s 5-alpha-reduced metabolites (5-alpha- pregnan-3,20-dion and 5-alpha-Pregane-3-ol-20-one) that are secreted by the CLs of ovarian vasculature in dominant follicle selection and ovulation. Previous research in other species has shown that changes in plasma progestagen changes do not coincide with observed changes in luteal structure. The purpose of this study was to perform a descriptive study that characterizes the relationship between luteal blood flow in growing and degrading CLs with the corresponding changes in plasma progestagen levels using color flow Doppler ultrasonography in Asian elephants. The study subjects consisted of six female Asian elephants ranging in age from 14 years to 50. The subjects were ultrasounded on a weekly basis for almost a year and a half. OvCL’s had larger amounts of vasculature than acCL’s (25+/-7%, 17+/-5%; t=2.928, df.30) but the number of days post ovulation at which the maximum blood flow occurred did not differ (28.81+/-15.22, 34.88+/-22.05; t=-0.905, df.30). The average maximum progestagen concentrations were 3.56 with a very large standard deviation of 3.83 and occurred at 29+/-10.84 days post ovulation. The ovulatory ovary had a higher maximum level of blood flow than the non-ovulatory ovary (21.4+/-3.8%, 15.1+/-4.74%; t= 4.480, df.30). The reproductive cycle was divided into the follicular and luteal phases and correlation between parameters were not detected. The luteal phase was subdivided into its three components of growth, mid, and regression phase. Within these subdivisions no consistent correlations existed. This study has shown that despite the fact that luteal blood flow, luteal area, and circulation progestagen follow similar distinct cycle patterns, no true correlation exist among the three parameters. It has shown that there are vascular differences between ovulatory ovaries and non-ovulatory ovaries that might suggest a role of ovarian vasculature in dominant follicle selection and ovulation.

Incidence of Reproductive Tract Leiomyoma in Indian Rhinoceroses

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Cited in husbandry guidelines and veterinary textbooks reproductive tract leiomyoma appears to be common in Indian rhinoceros. However, data on the incidence of leiomyoma in the captive population is scarce. In this study 15 female Indian rhinoceros 18.3 ± 2.4 years of age were assessed by ultrasound for their reproductive health and the incidence of reproductive tract tumors. Ten proven, four non-proven and one sub adult were examined. Proven breeders had reproduced their first offspring at 12.1 ± 1.1 years of age. Ultrasound assessment in proven breeders was conducted 7.5 ± 2.6 years after giving birth for the last time. The incidence of leiomyoma in proven and non-proven breeders >12 years of age was 100%. Average number and maximum size of tumors found was 15.4 ± 3.9 and 5.1 ± 1.1 cm, respectively. There was no significant difference in number or size of tumors between proven and non-proven breeders. However the incidence of tumors correlated strongly with age (r= 0.8411). Irrespective of the reproductive performance female Indian rhinoceroses seem to develop reproductive tumors during their lifetime. If leiomyoma development is inevitable in female Indian rhinoceros it is concluded that fecundity can be increased only by breeding this species at the earliest age possible. This conclusion is supported by data from the international studbook.

Session V: Veterinary Care

Manual Restraint and Chemical Immobilization With Xylazine/Ketamine of Wild And Captive Sumatran Elephants (*Elephas maximus sumatranus*) Under Field Conditions

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Conducting medical procedures in elephants often requires manual restraint or chemical immobilization. In modern zoos, sophisticated restraint devices have been developed for manual restraint and a high variety of different tranquilizers are available to achieve reliable and safe sedation and immobilization of elephants in these facilities. But more than 90% of captive Asian elephants live in the Asian range countries, where such sophisticated restraint devices do not exist, and many tranquilizers are not available due to legal restrictions, financial, and logistics limitations.

In Sumatra, as in many other range countries, safety during medical procedures relies on basic traditional tools and techniques for manual restraint of elephants. This includes different kinds of fore and rear foot hobbles, neck and body tethers made from different materials such as ropes, chains, and rattan, and the use of basic “homemade” restraint devices or just tree trunks as tether points. To properly apply restraint methods, the experience of mahouts is...
Standing Sedation in Sumatran Elephants (Elephas maximus sumatranus) Using Detomidine and Butorphanol

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Standing sedation was provided for comprehensive health and reproductive assessment and for minor clinical procedures in captive Sumatran elephants (Elephas maximus sumatranus). 33 (4, 29) animals were investigated within three days (11 procedures/day). The elephants (body weight ranged from 650 to 2.800 kg) were managed in free contact hence save access to ear veins was possible in all animal, except in one aggressive bull. However, none of the animals were conditioned to receive transrectal ultrasonography. Therefore standing sedation was imperative. An initial hand-injection of detomidine hydrochloride and butorphanol tartrate (mixed syringe) was administered intravenously (i.v., n=22) with a dosage of 10 - 40 mg/animal (10 - 25 μg/kg, mean 15 μg/kg) and 20 - 50 mg/animal (14 - 60 μg/kg, mean 21 μg/kg), respectively. The initial injection resulted in adequate sedation for initiation and completion of all procedures. No supplemental doses were required. The aggressive bull, not accessible for intravenous injection, received initially 60 mg (34 μg/kg) detomidine and 60 mg (34 μg/kg) butorphanol intramuscularly (i.m.) and a supplemental injection i.v. of 10 mg (5.7 μg/kg) detomidine and 20 mg (11.4 μg/kg) butorphanol after 15 min. Maximal effect occurred at 5 and 20 min after i.v. and i.m./i.v. application, respectively. All animals did not move at all and were standing steady with head down, trunk and tail 100% immobilized while they were able to keep balance without signs of ataxia. From the author’s experience from other Asian elephants (not included in this study) ataxia was observed with a dosage >25 μg/kg detomidine in combination with butorphanol > 40 μg/kg body weight. No cardiac or respiratory depression was appreciated. Reversals were applied 30 - 40 min after initial administration of sedatives. They were injected two-third and one-third intravenously and intramuscularly, respectively. Application of atipamezole (10 - 30 mg/animal, mean 8 μg/kg, SD 4 μg/kg) and naltrexone (12.5 - 50 mg/animal, mean 18.3 μg/kg, SD 8.1 μg/kg) resulted in rapid and complete recovery within 2 to 15 min. Animals diagnosed with kidney failures sonographically showed slower recovery (29 to 30 min). In contrast to former results were the authors used xylazine and yohimbine intravenously for short term standing sedation in Asian elephants in Thailand no adverse side effects on gastrointestinal tract (e.g. anorexia, abdominal distention or bloat) could be observed this time. The combination of detomidine and butorphanol is highly recommended for standing sedation in Sumatran elephants. In comparison to intramuscular administration of the sedatives total dosage and induction time was reduced when detomidine and butorphanol was administered intravenously.

Urinary Hormone Concentrations and Pharmacokinetics/pharmacodynamics of Haloperidol in a Female Indian Rhinoceros (Rhinoceros unicornis)

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Long-acting neuroleptics (LAN) are frequently used during immobilization and transport of rhinoceroses. A single administration of LAN reduces transport stress and aids boma acclimation in wild African black rhinoceroses. Long-term use (>3 weeks) of LAN’s have been reported in several non-domestic species, but have not yet examined in the rhinoceros. The goal of this study was to assess effectiveness of the long-term use of the LAN haloperidol to acclimate a 6.5 year old female Indian rhinoceros to novel stimuli. Haloperidol is unique in that it can be administered orally. Specific objectives included validating a urinary haloperidol assay to determine pharmacokinetics and pharmacodynamics over time and at different dosages. Urinary adrenal and gonadal hormone profiles were compared before and during treatment. Finally, behavioral correlates related to public exhibition and handling for reproductive assessment (transrectal ultrasonography) were evaluated.

Oral dosing of haloperidol was accomplished by inserting tablets (10mg) into a banana and hand feeding to the rhinoceros. During the first 50 days of treatment, the rhinoceros received 50mg PO. Thereafter, dosage was increased to 80mg PO. After 203 days, dosage was tapered for 34 days to

very important and veterinarians have to ensure that restraint devices are used and fitted without causing injuries to the elephants. Well trained koonkie elephants are an important “tool” often used to properly restrain and handle wild or untrained elephants. The effectiveness of a koonkie depends very much on the level of training and experience of the individual elephant and its mahout.

For chemical immobilization the only drugs reliably available on the market in Indonesia are Xylazine and Ketamine. These drugs have been proven to be sufficient for reliable standing sedation in captive and wild Sumatran elephants for different needs such as: light standing sedation for transportation, tusk trimming and wound treatment in unreliably trained animals, deep standing sedation for some basic simple surgical procedures like tail amputations and removal of tumors in combination with local anesthetics in captive elephants, and, in wild elephants fitting GPS collars, treatment of injuries, and translocations. Dosages used vary depending on the condition of the elephant and the level of sedation to be achieved.

In captive elephants we use dosages of 0.08 – 0.15mg Xylazine/kg BW combined with 0.03 – 0.06 mg Ketamine/kg BW by i.m. or i.v. injection. If prolonged sedation is needed in cases of time consuming treatments and surgery, a second injection with 1/3 to 1/2 of the initial dose can be administered about 60 to 90 min after the first injection, and in cases of light sedation (i.e. for transporting untrained elephants) about 2-4 hours after the first injection.

In wild elephants, dosages of 0.16 – 0.36 mg Xylazine/kg BW combined with 0.08 – 0.14mg Ketamine are used in cases of capture for translocation, fitting GPS collars, and treatment of injuries. In some cases 30 to 45 min after the initial injection, a second injection with dosages of 0.06 – 0.2 mg Xylazine/kg BW and 0.02 – 0.07 mg Ketamine/kg BW have been administered to achieve adequate tranquilization. Yohimbine is sometimes used as reversal about 45 to 75 minutes after the administration of the tranquilizer with a dosage of 0.05 – 0.11mg /kg BW.
complete discontinuation of treatment. Urine samples were collected daily. Urine haloperidol was measured using a commercially available enzyme-linked immunosassay (Neogen, Lexington, KY). The assay was validated for Indian rhinoceroses by analyzing urine from two untreated and one treated female (80mg PO). Samples were assayed in duplicate against blanks, a standard curve (0.008-5ng/mL) and controls. Urinary estrogen conjugate (EC; R522) and progesterone metabolite (PgG; R13904) concentrations were measured to monitor reproductive activity. In addition, urinary cortisol (R4866) was measured to reflect adrenal function.

No extrapyramidal side effects were noted during the 240 days of treatment. We found no difference (P=0.16) in background concentrations (0.76 + 0.01 ng/mg Crt; 0.13 + 0.01 ng/mg Crt) of haloperidol between Indian rhinoceroses and both were similar to background values reported in equine urine. Serially diluted urine from the treated female (80mg PO) demonstrated parallelism (r=0.99) to the haloperidol standard curve. There was no difference (P=0.32) in urinary haloperidol concentrations between 50mg (1.98 + 0.22 ng/mg Crt) and 80mg (3.08 + 0.19 ng/mg Crt) dosages. Both treatments were higher (P<0.05) than background levels. A dose dependent excretion effect (P<0.05) was observed during dosage decline, with urinary haloperidol concentrations averaging 4.07 + 1.08, 2.08 + 0.25 and 0.93 + 0.35 mg/mg Crt at 60, 40 and 20mg PO. Concentrations returned to background levels within 2 weeks of treatment ending. Of four estrous cycles that occurred during treatment, the female successfully ovulated once, which was comparable to previous reproductive outcome in this individual. Urinary cortisol concentrations during treatment averaged 50.37 + 1.63 mg/mg Crt and were higher (P<0.05) than those excreted 149 days prior (36.68 + 2.20 mg/mg Crt). A positive correlation (r=0.15; P<0.05) between urinary EC and cortisol was observed, underscoring the need to concurrently evaluate adrenal and gonadal hormone results in this species. This is the first data with regard to urinary pharmacokinetics/pharmacodynamics of the long-acting neuroleptic haloperidol in the Indian rhinoceros.

Acknowledgment: This study was supported by the P&G Wildlife Conservation Scholarship, Proctor & Gamble Pet Care, Cincinnati, OH.

Current Studies on Molecular Mechanisms of Iron Homeostasis in Rhinoceroses
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Iron storage disease (ISD) is a hazardous and clinically underappreciated condition commonly acquired by exotic wildlife species when displaced from their natural habitats and confined for even short periods under artificial conditions.

An international symposium recently reviewed and validated evidence that African black and Sumatran rhinoceroses invariably develop progressive ISD commensurate with their times in captivity, whereas African white and Indian rhinoceroses do not (1). Since vulnerability to ISD is a species-wide characteristic, it is likely to have a genetic basis possibly reflecting evolutionary adaptions to differences in iron bioavailability between browser and grazer diets.

As a biologically essential element that is also highly toxic in excess, iron is exquisitely regulated by molecular mechanisms primarily focused on interactions between the peptide hepcidin, (the principal iron-regulatory hormone), and its receptor ferroportin, (the sole channel for egress of intracellular iron into plasma) (2). Iron-regulatory gene sequences from both ISD-susceptible and non-susceptible species were compared to search for possible molecular differences. DNA was extracted from peripheral blood samples from all four available rhinoceros species, and genes encoding hepcidin and ferroportin, as well as modulators hemojuvelin, transferrin receptor 2, and HFE protein, were cloned and analyzed by PCR amplification. Over half of the DNA sequences continues, as do studies to determine the responsiveness of rhinoceros ferroportin to hepcidin modulation and quantitative levels of hepcidin expression (3).

In addition, liver and spleen mRNA sequences from African black and white rhinoceroses were assembled using Trinity RNA-Seq software (4) and compared with human sequences using the SIFT algorithm (5). Candidate single-nucleotide polymorphisms were independently validated by genomic sequencing. Mutations were found in four genes that may be associated with primary iron disorders or hemolytic anemia in black rhinoceroses: SLC28a2, EPB41, MTF1, and STEAP4 (6). The functional consequences of these mutations are being determined.

Literature Cited
Issues of Elephant Health Care Management in Myanmar Timber Enterprise (MTE) Myanmar
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Currently over 5000 captive elephants still belong to Myanmar; most of these elephants are owned by the government, and some are privately owned. In Myanmar the elephant situation is not so bad compared to our neighbor countries such as India and Thailand. Although, Myanmar elephants are still utilized for logging in the forest, most Myanmar elephants can live and spend their time freely during resting periods. On the other hand, according to MTE recorded data, the annual elephant death rate is higher than the annual birth rate. Working elephants in MTE are occasionally faced with work related injuries, wounds, swelling on the lower shoulder area, and tumors and fibrosis. According to fiscal year records of MTE, most old elephants die of malnutrition and old age, accidental cases, and a few from suspected infectious diseases. From 2011 to 2013, we notified that some baby elephants (less than 12 yr old) died with sudden death. The symptoms of these young elephants before death and necropsy findings after death were similar to those from elephant herpes virus. As prevention for bacterial infectious diseases, MTE elephants have to be inoculated with two kinds of vaccines: haemorrhagic septicemia vaccine (killed vaccine used in cattle) and anthrax vaccine (live spore vaccine special for elephants which is produced in Myanmar Livestock Veterinarian Department). Haemorrhagic septicemia vaccine is inoculated twice a year, and anthrax vaccine is inoculated once a year. Under MTE, there are 14 Region and State sub-departments. Under these 14 Region and State sub-departments, there are 43 agencies. Therefore elephants are distributed all over the country. But MTE has only 41 vets especially for elephants; they are veterinarians and para-veterinarians. The responsibilities of vets are not only in elephant health care management but also for wild elephants such as driving back to the forest to solve the problem of human-elephant conflict, and wild elephant translocation projects. The main causes of death of elephants in MTE, Zoos, and private owners are old age and malnutrition deficiency during summer season. The other causes are heat stroke, snake bite, accidents, diarrhea, parasitic infestation, malnutrition, bloat, bacterial infectious diseases, and illegal killing.

Update from the Stakeholders Task Force for the Management and Research Priorities of Tuberculosis for Elephants in Human Care
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There is historical and current evidence that elephants are susceptible to infection by MTB complex. However, only since 1996 have the elephant display and veterinary communities worked closely with the U.S. Department of Agriculture (USDA) to develop protocols for testing and treating elephants infected with MTB, and developed research priorities to learn more about potential risks and possible MTB transmission pathways (i.e. animal to animal, human to animal, and animal to human). There has also been an emphasis on putting the issue in context from both an animal and human health perspective.

In April of 2011, the USDA hosted a seminar at the Animal Welfare Information Center (AWIC) in Kansas City entitled: TB in Elephants: Science, Myths & Beyond. The meeting focused on issues of MTB in elephants, risk of transmission between elephants and to humans, and the role of new serological tests (commonly referred to as ElephantTB STAT-PAK® and MAPIA) in the detection of MTB in elephants. Presenters included representatives from USDA, NIOSH, CDC, University of Illinois, University of Georgia, Colorado State University, AZA, the Elephant TAG, Ringling Bros., the Tennessee Department of Health and several elephant researchers and veterinarians. Focus of the general discussion centered on issues related to diagnosis, treatment, and risk analysis.

At the conclusion of the seminar, Dr. Chester Gipson, Deputy Director of USDA-APHIS/Animal Care, encouraged interested stakeholders to further discuss the issues based on science and not politics and suggested a “stakeholders” meeting to further explore many of the issues raised over the two-day meeting, and to identify next steps.

The primary goal of the Management and Research Priorities of Tuberculosis for Elephants in Human Care Workshop is working with all elephant stakeholders to communicate and share what is known about tuberculosis in elephants and to focus on the science, treatment, research needs and regulatory issues associated with this disease.

Testing for Tuberculosis in Elephants: What is the Evidence?
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Infection with Mycobacterium tuberculosis in elephants is a source of concern, due to the organism’s potential impacts on public health, animal welfare, elephant conservation, and economic consequences. Diagnosis of tuberculosis infection in elephants has been difficult due to the insidious nature of the disease, and diagnostic limitations based on the animal’s size and various ante mortem testing methods. Accurate detection of tuberculosis infections...
in elephants will be an important factor in understanding the epidemiology and controlling the disease. Diagnosis is dependent upon the accuracy of diagnostic tests for this agent in elephants. There is controversy regarding the utility of currently available tests, particularly serology, and their proper role in the management of elephants with \textit{M. tuberculosis} exposure or infections. Tools for performing systematic reviews have been developed for resolving similar controversies in the medical care of humans. We performed a systematic review of literature pertaining to diagnosis and prevalence of \textit{M. tuberculosis} in captive and wild elephants. Our systematic review employed four different, but not mutually exclusive, review criteria for assessing the strength of study design and validity of results for each publication. This evaluation identified a number of weaknesses and limitations, including: small sample sizes; combined analysis of different host genera; incomplete differentiation of \textit{M. tuberculosis} from \textit{M. bovis} and non-Tb \textit{Mycobacterium} species; the limitations of the current “gold standard”, the triple sample trunk wash; and other study design flaws.

The degree to which the study design flaws that we identified affect the performance of diagnostic tests in target populations requires further investigation. Important concerns include the influence of disease prevalence on positive and negative predictive values (PPV and NPV, respectively) of diagnostic test results. For instance, the PPV decreases as the prevalence of disease decreases. Consequently, studies of elephant populations with artificially high prevalence may provide over-estimates of PPV for a given test. For populations where the prevalence of disease is low, even diagnostic tests with high specificity can yield unacceptably high numbers of false positives. Similarly, high disease prevalence populations may have low test NPVs and false negative results that are unacceptable. Failure to recognize the impact of prevalence on test results can result in incomplete identification of infectious animals, over-diagnosis, increasing treatment and management costs, and/or inappropriate euthanasia.

Many of these study design limitations are difficult to remedy, but must be acknowledged as a part of assessing risks of tuberculosis in individuals and populations of elephants. Candid and balanced assessments of diagnostic tests increase the likelihood that management strategies that are based, in part, on diagnostic test results will be effective for managing tuberculosis in elephants.

### Point Prevalence and Incidence of \textit{Mycobacterium tuberculosis} Complex in Captive Elephants in the United States of America

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Captive elephants infected with tuberculosis are implicated as an occupational source of zoonotic tuberculosis. However, accurate estimates of prevalence and incidence of elephant tuberculosis from well-defined captive populations are lacking in the literature. Studies published in recent years contain a wide range of prevalence estimates calculated from summary data. Incidence estimates of elephant tuberculosis in captive elephants are not available. This study estimated the annual point prevalence, annual incidence, cumulative incidence, and incidence density of tuberculosis in captive elephants within the USA during the past 52 years. We combined existing elephant census records from captive elephants in the USA with tuberculosis culture results obtained from trunk washes or at necropsy. This data set included 15 years where each elephant was screened annually. Between 1960 and 1996, the annual point prevalence of tuberculosis complex mycobacteria for both species was zero. From 1997 through 2011, the median point prevalence within the Asian elephant population was 5.1%, with a range from 0.3% to 6.7%. The incidence density was 9.7 cases/1000 elephant years (95% CI: 7.0-13.4). In contrast, the annual point prevalence during the same time period within the African elephant population remained zero and the incidence density was 1.5 cases/1000 elephant years (95% CI: 0.7-4.0). Accurate and species specific knowledge of prevalence and incidence will inform our efforts to mitigate occupational risks associated with captive elephants in the USA.

### Session VI: Veterinary Care - Viruses

**First Evidence Of EEHV Infection In Sumatran Elephants (Elephas maximus sumatranus) in Indonesia**

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We describe the first time an incidence of EEHV infection and mortality in the Sumatran subspecies of the Asian elephant (\textit{Elephas maximus sumatranus}) in Indonesia.

In 2005, a captive born elephant calf died at an age of about 2 years after a short illness lasting less than 24 hours, with unclear symptoms including anorexia, drowsiness, and some mild ataxia. Major gross pathological lesions observed internally were edema of the head and cyanosis of the tongue externally, and massive hemorrhage and petechiae on the heart, liver, lymph nodes, stomach, intestinal mucosa and sub cutis. Furthermore the liver was enlarged, the mesentery edematous, and large amounts of clear yellowish serous fluids were seen in the peritoneal and pleural and thoracic cavities and pericardial sac. At that time no proper laboratory facilities were available to run appropriate tests for infectious diseases. Discussing these findings later on
with international experts and the frequent appearance of small ulcers and blisters on the mouth mucosa of several adult elephants in several locations in Sumatra raised a suspicion of EEHV.

Seven years later, in April 2012, in a different location in Sumatra, 2 captive born calves died suddenly only 6 days apart, after showing no or very mild signs of discomfort only a few hours before death. Post mortem lesions were almost identical to the case from 2005. Two sets of tissue samples from all organs with pathological changes, and were collected and preserved in 96% ethanol and deep frozen at -20 C. Due to a lack of diagnostics for EEHV in laboratories in Indonesia the samples could not be tested immediately. About 6 months after collection of the samples a specific laboratory facility for molecular diagnosis of EEHV were established in Bogor. DNA was extracted from both frozen and alcohol preserved samples from heart, spleen and liver. Both cases were identified as EEHV 1 by conventional diagnostic PCR for PAN-EEHV POL and EEHV1-specific POL. These were then subjected to detailed gene subtype DNA sequencing at three key PCR loci, U38/POL, U51/vGPCR, U60/TER. These two cases have identical EEHV1A DNA sequences to one another indicating a common epidemiological source.

Clinical Signs, Diagnosis, and Treatment of the First Clinical Case of EEHV3B in an Elephant
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Elephant Endotheliotropic Herpesviruses (EEHVs) are ubiquitous in both Asian (Elephas maximus) and African (Loxodonta africana) elephants. While it is well recognized that some EEHV types such as EEHV1 can cause lethal hemorrhagic infections in juvenile Asian elephants, there is comparatively little information on the ability of the other EEHVs to cause disease, especially in African elephant calves. Here we report for the first time that EEHV3 can cause significant clinical disease in a juvenile African elephant. A 5 year old male African elephant presented with mild stiffness in the front leg, lethargy, and reduced appetite. A complete blood count (CBC) revealed leukopenia with a left shift, mild anemia, and high numbers of platelets. Treatment was started with oral famciclovir, as well as oral and rectal fluids. Polymerase chain reaction (PCR) revealed Elephant Endotheliotropic Herpesvirus (EEHV) 3 in whole blood taken at the time of clinical presentation, and genotyping identified the virus as EEHV3B. Quantitative PCR showed viremia as high as 100,000 VGC/ml of blood, which slowly decreased to undetectable levels over the following 35 days of treatment. During the course of the disease, the white blood cell count rose from the initially low level on day 2 to an elevated level until the third week, when the value returned to the animal’s normal range. Concentrations of acute phase proteins (serum amyloid A and C-reactive protein) and beta globulins rose initially as well before returning to normal levels after several weeks. During the second week, the calf developed a single vesicle on the tongue and edema on the top of the head, which resolved over the following two weeks. Therapeutic intervention at the first sign of disease, as well as frequent monitoring of CBC, urinalysis, protein electrophoresis (EPH), and acute phase proteins are likely to have contributed to the success of this case.

Seven Species of Elephant Endotheliotropic Herpesviruses (EEHVs) Form a Novel Mammalian Subfamily the Deltaherpesvirinae
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Extensive genetic analysis of elephant endotheliotropic herpesvirus (EEHV) genomes from infected Asian and African elephants has both greatly clarified the evolutionary relationship between the Proboscivirus genus and other mammalian herpesviruses and revealed multiple distinct species and subtypes. In particular, the complete 177-kb genome of the most highly pathogenic species EEHV1A (strain Kimba) had been compiled from infected Asian elephant necropsy tissue. This data has revealed that 64 of the total of 115 genes are novel, including 20 members of the vGPCR family, five IgFam genes, two captured cellular glycosyl tranferases and three vOX2 genes. Analysis of another two strains each of EEHV1A, EEHV1B and EEHV2 (totaling 60 to 65-kb each), as well as three strains of EEHV5 and two of EEHV6 (totaling 25 to 30-kb each) have revealed that all five AT-rich branch EEHV types (which diverge from each other by 15 to 20%) have an inversion of a 40-kb core segment of the genome relative to betaherpesviruses. Three other species EEHV3, EEHV4 and EEHV7 (4-kb each) are even more highly diverged (35%) and form a distinct GC-rich branch of the Proboscivirus genus. The AT-rich branch EEHVs also all encode GPK genes, plus alphaherpesvirus-like TK, RFB, OBP genes and an Ori-Lyt domain that are absent from betaherpesviruses. Most dramatically, all EEHV genes and proteins encoded in common with other herpesviruses are at least 50 to 80% diverged from their nearest orthologues. In both DNA and protein based phylogenetic trees the EEHVs fall into a monophyletic clade branching intermediate between the mammalian gammaherpesvirus and betaherpesvirus sub-families. Therefore, we propose that the Probosciviruses (=EEHVs) should be designated as the prototypes of a new Deltaherpesvirinae sub-family, which we estimate has evolved separately from the three other mammalian Herpesviridae sub-families within Afrotdian hosts, including the ancestors of modern elephants, for more than 100 million years.
elephant herpesviruses include Probosciviruses EEHV1A, EEHV1B, EEHV2, EEHV3A, EEHV3B, EEHV4, EEHV5A, EEHV5B, EEHV6, EEHV7A, EEHV7B and Elephant Gammaherpesviruses EGHV1A, EGHV1B, EGHV2, EGHV3A, EGHV3B, EGHV4, EGHV5A, EGHV5B. Herpesviruses generally are species-specific, but if inter-species infection occurs, severe pathology in the non-natural host may be the outcome. It was hypothesized that herpesviruses of African elephants were infecting Asian elephants, causing acute disease and hemorrhagic death, so it was crucial to identify which herpesviruses are endogenous viruses of African elephants. In 1996, viral particles morphologically consistent with herpesviruses were found in nodular raised fibrous cutaneous lesions biopsied from African elephants imported from Zimbabwe to America. In 2009, corresponding author Virginia Pearson observed similar nodules on wild African elephants in Kenya, and, in 2011, led an expedition in Kenya to obtain biopsies from these skin nodules. In collaboration with Save The Elephants and Kenya Wildlife Service Veterinary and Capture Services Department, we immobilized twelve wild elephants in Samburu and Maasai Mara National Reserves. We collected saliva, blood and exudates from all twelve elephants, biopsies from raised cutaneous nodules on five of the elephants, and lung biopsies from a thirteenth recently dead elephant. By extensive polymerase chain reaction (PCR) and viral DNA sequencing analysis at Princeton University and subsequently at Johns Hopkins School of Medicine, we identified DNA from EEHV2, EEHV3A, EEHV3B (a new subspecies), EEHV6, EEHV7A and EEHV7B (a new subspecies) in skin nodule and lung biopsies and saliva from these wild African elephants. Also, we have found EEHV2, EEHV3A, EEHV3B, EEHV6 and EGHV1B (a new species), EGHV2 and EGHV4 DNA in saliva collected weekly for one year from two captive asymptomatic wild-born African elephants in America, and in saliva samples collected occasionally from an additional two dozen captive African elephants. No EEHV1A, EEHV1B, EEHV4 or EEHV5 DNA sequences have been found in these wild or captive African elephant skin or lung biopsies or saliva samples. Our findings of DNA sequences, together with PCR sequencing from necropsy lung nodule tissues from two culled South African adult elephants and an adult African elephant euthanized in the United States have shown that the subset of Probosciviruses EEHV2, EEHV3A, EEHV3B, EEHV6, EEHV7A and EEHV7B, but probably not EEHV1A, EEHV1B or EEHV4 or EEHV5 are likely to be natural endogenous viruses of all African elephants. We conclude that cross-species infection between the two elephant genera is extremely rare and does not account for the unusually severe pathology and hemorrhagic deaths in juvenile captive and wild African elephants. EEHV2 and EEHV3 have caused hemorrhagic deaths in two captive African and one captive Asian elephant respectively. However, it is yet to be confirmed whether the presumed endogenous herpesviruses of African elephants cause hemorrhagic deaths in wild African elephants, as has been shown for EEHV1A, EEHV1B and EEHV4 in wild Asian elephants and EEHV5 in one captive Asian elephant. Whether co-infections with Elephant Gammaherpesviruses (EGHVs) affect pathogenesis of hemorrhagic disease in African and Asian elephants is also yet unknown.

**Elephant Endotheliotropic Herpesvirus (EEHV): Where We Are, Where We’re Going**

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Elephant endotheliotropic herpesvirus (EEHV) is the single largest cause of death in Asian elephants (Elephas maximus) born in North America since 1978. In January 2013, the Houston Zoo and the International Elephant Foundation hosted the 9th annual International EEHV workshop, which focused on recent EEHV research and prioritizing needs for the future. Highlights from workshop presentations include:
1. That the EEHV1 genome has been fully sequenced along with partial sequencing of other EEHV types and collectively, based on their significant divergence from other known herpesviruses, should be classified as a new subfamily of Deltaherpesviruses.1

2. That there are currently at least 11 subtypes of EEHV, which, with minor exceptions, are endogenous to Asian elephants (EEHV 1, 4 and 5) and African elephants (EEHV 2, 3, 6, and 7).

3. That healthy African elephants in North America have been shown to shed EEHVs from their trunks, and more information is needed on EEHV in African elephants

4. On preliminary investigations into the immunity of elephants related to EEHV, with much more study needed on this important topic.2

5. On clinical management of EEHV-associated illness in affected elephant calves.3, 4

References:


Veterinary Treatment and Management for Papiloma Viral Infection of a Captive Sri Lankan Elephant (Elephas maximus maximus) In Colombo Zoological Garden

The Sri Lankan elephant (Elephas maximus maximus) is an endangered species in IUCN categories. A new census revealed that the country has around 5800 wild elephants and 213 captive elephants. Due to human-elephant conflict-related reasons, on a daily basis elephants deaths are reported. Out of the captive elephants 86 are in the Pinnawala Elephant Orphanage and 7 are in the National Zoological Gardens in Colombo. Among captive elephants most of them are reaching beyond a young age. Some of them have health problems that are age-related. According to their age, immunity deficiency has occurred and some infectious diseases have been observed. Papiloma viral infection, on the foot, is observed mostly in old elephants, but occasionally in young elephants that had some immune deficiency in captivity. It is a serious health issue and it can damage the foot and simultaneously can incur a pododematitis condition. Severe growth with a bacterial infection leads to damage of the foot and creates serious health problems with the foot as well as with the life of the elephant.

Colombo Zoological Gardens has seven elephants, six females and one old male on location. ‘Devi’ is a female elephant, 24 years old, with an average body weight of 4000 kg. She was used for elephant performances during the last 17 years. Recently, due to an arthritis condition, she was retired from elephant performances. She was infected by a papiloma viral growth on the foot of the right hind limb. It causes limping and shows a clearly visible flower-like growth on the lateral margin of the foot of the right hind limb. Initially a sample from the growth was removed and sent to the Veterinary Research Institute for confirmation. According to the clinical signs and nature of the lesion, treatments were started. The elephant was trained for foot dipping, by putting a leg into a medicated foot bath which contained 10 L of water mixed with 500 ml of Providone iodine and 2 g of KMNO4 (Potassium Permanganate) and by keeping the affected leg in the foot bath for 15 minutes. After cleaning the area of the lesion a combination of which contained 5 g CuSo4 (Copper sulphate), 5 Aciclovate tablets powder (anti-viral tablet), and Base of Stalkhalm Tar. The mixture was applied directly on the lesion and the stalkhalm tar was applied to the rest of the margin of foot. For the bacterial pododemattes, she was given a Penicillin Streptomycin Intramuscular injection 80 ml at three day intervals for up to two weeks. She was also given vitamin A, D and E injections two consecutive times at 5 days intervals. The laboratory sample confirmed it as a papiloma viral growth. For preventing the spread to others, the elephant was relocated to a corner, but not completely separated because stress can lead to a drop in the immune response. The elephant was teathered in place to a much drier environment and was fed oral calcium. With treatment for 35 days the growth gradually decreased and was completely cured. Throughout this period to maintain the elephant's immune system, on a daily basis ‘Devi’ was fed 25 tabs of 100 mg Ascorbic acid, 30 Vitamin B complex tabs, and 10 Folic acid tabs. ‘Devi’s’ papiloma growth was completely cured and for future prevention, Providone iodine was sprayed on the foot twice weekly.
Studying anthropomorphism toward solitary animals can help zoos address concerns about animal welfare and determine appropriate educational responses.

A random sample of 200 guests were asked to complete a survey on 1 of 4 black rhinoceroses (Diceros bicornis) located at Brookfield Zoo's Pachyderm House. The topics on the survey ranged from the rhino’s behavior and welfare to the guest’s environmental viewpoints and their emotional connection to the animal. Survey responses were examined for awareness of the black rhinoceros’ solitary lifestyle, satisfaction with the animal’s enclosure, and anthropomorphic descriptors. Correlations were investigated with SPSS software.

Analysis revealed 64% of survey respondents were unaware of the black rhinoceros’ solitary lifestyle. When perceived as social, guests were more inclined to agree that the rhino appeared lonely (Spearman’s rho, \(r_{(144)}= 0.186\), \(P = 0.026\)), to disagree with the rhino not needing a companion (Spearman’s rho, \(r_{(145)}= 0.345\), \(P = 0.000\)), and to agree that the rhino seemed stressed with the presence of visitors (Spearman’s rho, \(r_{(145)}= .166\), \(P = 0.046\)). When describing the rhino’s mood, 15% of guests used negative anthropomorphic descriptors i.e. “lonely”, “sad”, “bored”, “depressed”. When describing their own emotional response to the animal’s behavior, 2% of guests used negative descriptions i.e. “felt sad for the rhino”. The rhino’s activity level, exhibit space, and enrichment items also influenced the guest’s perception of the animal’s welfare.

These findings suggest that educational outreach should be increased to improve guest awareness and satisfaction with solitary animal exhibits. Otherwise, zoos will need to determine how to reconcile visitor preference for multianimal exhibits with the black rhinoceros’ solitary lifestyle.

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Session VII: Ex situ Benefits and Support of In situ Conservation


Sean Hensman
Elephants for Africa Forever

We’re living in an imperfect commercial world which is constantly evolving and modernising. Humans are continually putting pressure on wildlife. Ultimately everyone has the same conservation objective; to ensure that our grandchildren’s grandchildren are able to experience wildlife better than we currently do, we all just have differing opinions on how to achieve this.

Having had a rich wildlife and conservation orientated upbringing Sean Hensman, from Adventures With Elephants in South Africa shares his family’s story on how their tamed and trained elephants are wildlife ambassadors contributing to the wider conservation of elephant. This ranges from interactive and educational tourism, promotional aspects, research on a variety of issues, elephant welfare and finding future potential by harnessing elephant’s incredible sense of smell to detect explosives and track people. All these different capabilities give elephant’s incredible value, thus ensuring their survival in our future imperfect world.

Contributions to Science and Conservation by Elephant Managers and Captive Elephants

Heidi S. Riddle
Riddle’s Elephant and Wildlife Sanctuary, Arkansas and Elephant Managers Association

Captive elephant management has greatly evolved since elephants first came to North America in the late 1700s as part of menageries exhibiting unusual animals. In early zoological management, an elephant keeper’s main concern was basic animal care and husbandry; in present times, however, captive elephant managers and their elephants play a significant role in the scientific study and conservation of the species.

In 1988, the Elephant Managers Association (EMA) was established in the United States, and in 2006 the first professional association for elephant managers in Asia was developed in Indonesia as the Sumatran Mahout Communication Forum (FOKMAS). Both groups provide an opportunity for elephant keepers/handlers/trainers (mahouts) to come together, share experiences, and improve elephant management. Captive elephant managers have many roles: we provide care to the elephants, act as educators, conduct some public relations, contribute to scientific research, and we raise awareness, funds, and work to support the conservation of wild elephant populations.

Intensive scientific studies of the captive elephant population over the past 20 years have taught us much about the biology and physiology of the animal. Elephant managers are instrumental in biological studies and elephant health care work – managers train and condition elephants to veterinary procedures such as blood collections, trunk wash sampling, ultrasound or radiographic assessments, as well as for medication routes (i.e. oral, rectal). Without the cooperation and input of elephant managers and the ability to obtain samples for diagnosis, scientists would not be able to study diseases of concern to elephants (i.e. EEHV), and wildlife veterinarians would not be able to implement effective elephant health care programs.
Captive elephant management is an integral part of elephant conservation. Almost one third of the Asian elephant species is in captivity, and most of those animals are in Asia. Captive elephants in the western world and in Asian range countries are kept in a variety of environments; much can be learned from these different situations about elephant care, husbandry, and training. Working together with other elephant managers, groups such as EMA and FOKMAS have not only improved elephant husbandry, but also make important contributions to the scientific study and conservation of these animals. Everyone in the elephant community (both wild and captive) needs to work together to learn from each other and help improve all aspects of elephant management; only then will we truly be successful in our work with and for elephants.

Contributions of the Ringling Bros. Center for Elephant Conservation to Wildlife Management in Sri Lanka
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2 Ringling Bros. Center for Elephant Conservation, Polk City, Florida, USA
3 Center for the Study of Asian Elephant at Rajarata University of Sri Lanka at Mihintale, Sri Lanka

In order to enhance the management of wildlife in general and the elephant in particular in Sri Lanka, the Ringling Bros. Center for Elephant Conservation® (Ringling Bros. CEC) in Polk City, Florida, USA hosted four Sri Lankan graduate students for one year of training and research, utilizing elephants at the Center, as part of their postgraduate degree program with the University of Peradeniya, Sri Lanka. Three of them have submitted their theses for review, while the fourth student’s research was upgraded to PhD. Ringling Bros. CEC subsequently built a bullpen for the Temple of the Tooth in Kandy to manage bulls in musth without them being chained. The partnerships with Sri Lankan universities, the Department of Wildlife Conservation (DWC), and Ringling Bros. CEC led to the surveys of human-elephant conflict in 2008, 2009 and 2011. Ringling Bros. CEC has also established the Ringling Bros. Center for the Study of Asian Elephant at Rajarata University in Mihintale, Sri Lanka and provided funds for student projects. In addition, it has provided resources to conduct a course on Wildlife Conservation & Management at Rajarata University. At the Elephant Transit Home (ETH) in Uda Walawe National Park, Ringling Bros. CEC has been assisting the DWC in monitoring the growth of the orphaned elephant calves by weighing them at monthly intervals for over three years. The data from weighing these calves has become a unique resource. In addition, the program is also assessing the changes in the body condition of wild elephants at monthly intervals. An accurate method to estimate the length and/or height of elephants and other wildlife using laser beams has been introduced. In addition, the decibel levels of the firecrackers that villagers use to ward off wild elephants were tested and found to be very high indeed. Frequent bursting of such firecrackers in close proximity to elephants may impair their hearing. An innovative method to monitor wild elephant movement was initiated using standing sedation during which a home-made GPS/GSM collar was affixed to the bull. The elephant’s movement was then tracked on line once every minute for a month. This technology was mainly developed to monitor marauding elephants with the aim of mitigating the human-elephant conflict. Ringling Bros. CEC assisted the DWC in the planning and execution of the first National Survey of Elephants in Sri Lanka in August 2011. It also carried out conservation education programs at several schools and colleges in the island. Students from Missouri State University in USA came over to the Ringling Bros. Center for the Study of Asian Elephant at Rajarata University in the summer of 2012 to observe wildlife (including elephants) and agriculture and to gain an understanding of the culture where elephants are a part of the landscape.

Employing Mahouts and Captive Elephants for Elephant Conservation Programs in Sumatra
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2 Forum Komunikasi Mahout Sumatra, Indonesia
3 Veterinary Society for Sumatran Wildlife Conservation, Medan, North Sumatra, Indonesia

In Sumatra, the management of captive elephant populations and professional mahouts is fairly recent, and only exists since the mid 1980s when the Indonesian government launched a program to capture wild elephants in Human Elephant Conflict (HEC) areas and brought them into captivity for domestication. Due to unsatisfactory concepts about how to utilize the captured elephants, a lack of sufficient resource for the care and management of these elephants, and a lack of sufficient training and education for the newly recruited mahouts, the management and care of these elephants was initially lacking in many ways.

To address these issues and involve captive elephants and mahouts in elephant conservation strategies, over a decade ago some NGOs, along with government conservation agencies started to develop concepts and programs to establish elephant patrol units where mahouts are trained and employed, and captive elephants are utilized for wild elephant conservation activities. These activities include HEC mitigation and management by monitoring wild elephants and their movements, driving them away from cultivated areas and back into protected forests, or in cases where single elephants have ventured deeper into farmland or settlements evacuate these animals and relocate them back into protected forest areas. Further activities for which mahouts and captive elephants are sometimes employed include the translocation of wild elephants from encroached areas to suitable habitats, education awareness activities, and fitting GPS collars on wild elephants for monitoring their movements to evaluate home ranges, habitat utilization, and HEC management prevention.

During the past years an increasing number of mahouts have been trained and more elephants are being utilized in such patrol units in many provinces in Sumatra. These units are mostly managed in collaboration between conservation NGOs and government conservation agencies. These units significantly contribute to conservation needs for wild elephants and their habitat, and, in areas such as the Way Kambas National Park, have become an important part of the government’s HEC mitigation strategies.
To successfully implement these described activities the skills and experience of the mahouts, who in the past were involved in wild elephant captures, is absolutely crucial. They have a very good knowledge about wild and captive elephant behavior and handling in such situations. The training given to these mahouts about field navigation, conservation needs, and regulations in combination with their elephant handling skills have made them valuable and effective field conservation workers for elephant conservation in Sumatra.

Reproductive Assessment of Sumatran Elephants (*Elephas maximus sumatrensis*) in Elephant Conservation Centers (ECC) Across Sumatra

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The Sumatran elephant is a subspecies of the Asian elephant and listed as critically endangered by the IUCN Red list. Besides a wild population of currently 2000-2500 elephants, there are about 650 elephants in captivity. These were mostly wild caught between 1985 and 2000 in order to reduce human elephant conflicts (HEC). The majority is maintained in governmental Elephant Conservation Centers (ECC) across Sumatra. A few elephants are also utilized in Conservation Response Units (CRU) in order to fight illegal logging and poaching.

Fragmentation of habitats, human elephant conflicts (HEC), habitat encroachment, and the illegal killing of elephants, diminish the wild Sumatran elephant population. Utilizing the captive elephants for various conservation activities and programs, and ensuring its long-term survival as a self-sustaining genetically valuable population, will back up wild elephant conservation strategies. To address these strategies, the careful management of the captive population is crucial, requiring ongoing professional veterinary care, consideration, and management support. The Veterinary Society for Sumatran Wildlife Conservation (Vesswic) started its Elephant Health Care Program (EHCP) in 2006 to provide veterinary expertise for Sumatran elephant conservation in Sumatra. Regular visits of 10 different camp locations, ensure basic veterinary care such as diagnostics and treatment, providing drugs (e.g. antibiotics, dewormers, tetanus vaccination), management and nutrition, foot and wound care (Stremme et al., 2007).

However, to ensure that the valuable genetic pool of the captive Sumatran elephants is represented, breeding of these animals is a priority:

- To ensure the availability of sufficient numbers of healthy elephants to be utilized long-term in programs such as CRU or HEC mitigation and without relying on further capture of wild elephants
- To ensure the long-term existence of captive elephants to function for the public as ambassadors of wild elephant conservation
- As back up for wild populations, e.g. by reintroducing captive individuals or small groups into smaller isolated wild populations to increase genetic variation and thus improve their odds for long-term survival
- For reintegration into rehabilitated habitats having lost their wild populations, e.g. previously logged forest concessions with secondary forest growth

However, in the past 10 years, there have only occurred 30 births, of which 22 offspring survived (73.2 %). In order to enhance breeding and to be able to monitor the reproductive status of the camp females, VESSVIC implemented ultrasound to their veterinary repertoire. Here we summarize the results from a first survey on the reproduction of the Asian elephants in Sumatra in order to assess the breeding potential.

Basic breeding data

During 2002-2012, a total of 30 births (13 males/16 females/ 1 unknown sex) from 27 different dams were recorded. Of these, 22 calves are presently still alive. Of the 8 calves that died, one was stillborn, 3 died to EEHV in 2 different camps (two cases PCR confirmed), one was killed after birth by the dam and 3 deaths occurred within days or weeks after birth for unknown reason.

If natural breeding occurs, it is usually by wild bulls, because captive bulls are always tethered and social interactions are thus very limited. However, in cases were males or females broke loose or the chains were long enough, also captive bulls were seen mating. In one camp (Tangkahan), an electric fence was established which allowed a male and six females to socialize during the day. This resulted in mating and 3 of the six females became pregnant between 2009 and 2012.
<table>
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<th>ETC Saree</th>
<th>CRU Tangkahan</th>
<th>BBS</th>
<th>ECC Way Kambas</th>
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* females younger than 5 years not included

Table 1. Overview of elephant examined in the 6 different camps

During birth, elephants were chained in 25 cases by one hind leg chain. In two camps, birthing paddocks were built and used in 4 cases. No group birth occurred and as of yet, there is no dystocia reported. Mahouts usually found the calf the next morning, only in a handful of cases, mahouts were able to witness a birth.

Ultrasound Assessment

A portable, battery driven ultrasound device (Micromaxx, Sonosite, Fa. Frings Medizintechnik, Germany) equipped with a 2-5 MHz convex probe was donated to VESSWIC in 2012 (through funds from U.S. Fish and Wildlife Service and Asian elephant Support). This US machine is very durable and has an average battery lifespan of 5 hours. Thus, being an ideal tool for the field situations in Sumatra. For the examinations, elephants were tethered to a tree or, when available, within a restrainer (Fig. 1). The rectum was cleaned and an enema given with a hose pipe.

In two trips, a total of 49 elephants were assessed via ultrasound in six different locations from Aceh to Lampung province, of these 32 were females (table 1).
Female ultrasound assessment

During assessment, we found a total of 7 pregnancies. These pregnancies were detected by transrectal ultrasound (3 cases) or transabdominal ultrasound (4 cases). Further indicators of pregnancy were large corpora lutea (CLs) on one or both ovaries, a mucus plug within the vagina and bright cervical folds. The mean number of CLs found in pregnant elephants was 2.8 CLs on both ovaries. This is markedly lower than the number of CLs reported for pregnant African (average of 6 CLs; Allen, 2006) or pregnant, mainland Asian elephants (average of CLs: 5.4; Lüders et al., 2010). However, the CLs were usually quite large, measuring 3.5-4.5 cm in diameter. From the non-pregnant elephants, 11 females were cycling and showed a healthy reproductive tract, five were in lactational anestrus with a calf (7-16 months) at foot, four were prepubertal and seven showed pathologies or acyclicity, thus considered post-reproductive (table 1). The ovarian medulla of most elephants showed distinct hyperechoic spots (indicative of dense, possibly fibrotic tissue), but this was irrespective of reproductive status.

Four prepubertal females (age 5-11) were scanned. There was a large mucus plug within the vagina, the uterus appeared dark and no functional structures were seen on the ovaries.

Of the cycling females (mean age: 24 years, range: 12-39 years), 8 were in the luteal phase and only 3 in the follicular phase, with one cow in estrus with a dominant follicle measuring 21.0mm. During the follicular phase, follicles of different sizes and small CLs (mean number: 2.5 CLs) were present, while the uterus appeared bright and convoluted. During the luteal phase, larger CLs, but no follicles were observed. The uterus was usually not visible after the bifurcation, due to its relaxed status.

The seven post reproductive females aged 33-45 years (mean: 38 years) and showed no ovarian activity (n=4) or ovarian activity, but reproductive tract pathology (n=3) such as uterine leiomyoma, cysts in the uterus or ovary, cervical leiomyoma or a combination of these pathologies.

Male ultrasound assessment

Bull elephants examined aged between 10 and 28 years. The lower reproductive tract organs, such as bulbourethral glands, prostate, ampullae and seminal vesicle were evaluated. Testicles could be reached with the hand held ultrasound probe or with the help of a plastic extension (older, larger bulls). In all but one bull, the seminal vesicles yielded no fluid filling, the ampullae were generally small and anechoic, the prostate not distinct and the testicles rather small (10-15cm in diameter).

Discussion

The overall reproductive parameter in Sumatran elephants investigated in this survey resembled those described for mainland Asian elephants. Remarkable was the lower number of corpora lutea (usually 2-3 CLs) and the prominent hyperechoic spots found in the ovaries of mature cows. These may be fibrotic foci, however their meaning and origin are unknown.

Of all females scanned, ranging from 5 to 45 years, only 7 females were considered post-reproductive due to uterine or ovarian lesions or acyclicity. However, the majority of females is reproductively healthy and still young enough to breed. Eleven females were showing ovarian cyclicity (four of these previously had calves) with another four females prepubertal and five in lactational anestrus. Therefore, in the potential breeder (n= 11) and proven breeder group (n=16) we found a total of 27 elephants ranging from 5-33 years. Thus, 79.4 % of the females in this survey are considered fertile and may reproduce.

Of all non-pregnant females, only four were acyclic for an unknown reason. This is 11.8% of females investigated and is close to the 14% of acyclic Asian elephant females recorded in North American zoos aged >30 years (Brown et al., 2004). Although malnutrition, parasite load and distress related to a lack of social interactions, constant tethering, and the inbreaking procedure through the mahouts, are obvious, acyclicity has only been observed in four elephants. This occurred exclusively in the older age class (>33 years). The low fecundity of the captive population appears therefore not related to fertility issues. The main problem is likely the lack of undisturbed interaction possibilities for these elephants, thus lack of breeding opportunities. This is a management issue. With the ultrasound at hand now, it will be possible to determine which females should be given opportunity to be breed. As persuaded in Tangkahan, paddocks for temporary mixing of elephants would be ideal.
Another problem may be the suppression of males in a captive situation. Reportedly, males adapt not as good to the captive environment compared to the females. Also too many bulls are kept within close vicinity in some camps (e.g. Way Kambas, were about 30 males are kept). Fighting and injury, sometimes even fatally, occur between bulls that broke loose or were chained to close together. Due to the presence of more than one adult bull as well as the poor nutritional status, most males appeared reproductively suppressed. This was reflected in the overall small testicle size and diminished filling of accessory sex glands. In result, only few males will be capable of breeding in these camps.

In conclusion, breeding needs to be promoted in order to keep the genetic diversity in the ECCs at high level and to establish a self sustaining captive population. Mahouts must be educated as to when allow males to have access to females. Birthing and mating paddocks need to be established in each facility.

Although the number of births did increase during the past four years, there is a higher potential in the captive Sumatran elephant pool.

Acknowledgement

The authors would like to appreciate the grants through the Asian elephant fund of the United States Fish and Wildlife Service as well as the Asian elephant support, which allowed to purchase the equipment.

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What Elephants Can Teach Us About Preventing Cancer

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Peto’s Paradox describes the puzzling observation that the increased cellularity of larger animals does not correlate with the expected increase in cancer risk. This is particularly true in elephants where there is only a 4% cancer incidence compared to the 30% seen in most other mammals, including humans. Evolutionary biologists have proposed one explanation for this paradox in which better functioning DNA repair mechanisms may provide larger, long-lived mammals with an improved ability to counteract cellular stresses that may cause mutations. One gene that may contribute to this increased DNA repair robustness is the known tumor suppressor gene, TP53. TP53 has been called the “guardian of the genome” and counteracts DNA damage by-initiating DNA repair or alternatively by causing damaged cells to undergo cell death (apoptosis). While most organisms encode only one copy of TP53, African elephants encode this copy as well as 18 additional copies of the TP53 gene. These additional copies in elephants appear to be retrogenes, which are ancient copies of the original TP53 gene that were incorporated into the elephant genome over evolutionary time. These extra copies of TP53 may play a role in elephant cancer resistance by initiating a hyper-repair or apoptosis response to more efficiently block mutations from propagating throughout cellular generations. To investigate this possibility, African elephant and human radiosensitive peripheral blood mononuclear cells (PBMCs) were exposed to DNA damage by gamma irradiation (IR) and compared in vitro for cell viability, DNA repair, cell cycle regulation, and apoptosis at subsequent time points. This study revealed a significant linear increase in apoptosis in elephant PBMCs exposed to 2 Gray γ-IR compared to human (p<0.005) which suggests that the cellular threshold of DNA damage in elephants is lower than in humans. These results indicate that elephant PBMCs preferentially undergo cellular death instead of DNA repair following IR exposure, and do so at a higher rate than humans. This mechanism of action may contribute to elephant cancer resistance by preventing mistakes in replication through rapid elimination of damaged cells which could otherwise lead to cancer.
The Critically Endangered Sumatran rhinoceros (*Dicerorhinus sumatrensis sumatrensis*) is one of the most threatened of all land mammals on Earth. Less than 100 Sumatran rhinos remain, primarily on Indonesia’s Sumatra Island, where the population has declined at a rate of 50% over the past 10 years, largely from poaching, deforestation and habitat fragmentation. They live in three important protected areas in Sumatra: Way Kambas National Park (WK) in Southeast Sumatra, Bukit Barisan Selatan National Park (BBS) in Southwest Sumatra and Gunung Leuser National Park (GL) in North Sumatra.

The Rhino Protection Unit (RPU) has been operating in this area since 1997, through intensive anti-poaching and intelligence activities to patrol forests and monitor endangered species, destroy snares and traps, apprehend poachers and prevent encroachment. There are seven units of RPU in BBS operating effectively within South Sumatra. As a result of these operations over the past 11 years, there have been no cases of rhino poaching encountered, and the rhino population seems to be increasing (estimates from 25 to 40) in Bukit Barisan Selatan.

The BBS NP is surrounded by about 12 million people who live in and around forests with a thin buffer zone, and many more are dependent on coastal resources. Forest conversion to agriculture increases the interface between humans and wildlife, which increases the opportunity for conflicts as competition for precious resources escalates. As a result, there is a high level of human-wildlife conflict. The poorest rural people are most dependent on biodiversity and natural habitats for their livelihoods, and they are the ones who suffer most when such habitats are simplified, degraded or otherwise impoverished. These people hold the future in their hands — they will either help save the rhinoceros or help move it toward extinction.

Some villages around NP are recognized as “problematic” as many of the poachers and encroachers operate from these problematic villages. To address the threats posed by some of these areas, the national park authorities supported by some NGOs have conducted several community development programs with no significant success due to difficulty in accessing those areas. However, the RPU has been regularly visiting the areas, staying for some time while performing their activities. Through these interactions, the RPU has earned the respect of the villagers.

Literature studies, surveys and consultations with agriculture experts were conducted by the RPU before they started to facilitate the villagers. The RPU realized that cacao is a more sustainable agriculture crop than coffee (e.g., cacao, has higher yields and brings higher prices). Unlike coffee, cacao is significantly more environmentally friendly, does not require land clearing or massive irrigation, and can be grown as part of a mixed natural forest system in park buffer areas. Before this idea was proposed to the villagers, the RPU conducted some experiments planting cacao in their personal gardens.

As a result of these activities, the local villagers were persuaded to surrender more than 90 illegal arms to RPU and NP authorities which is indeed an encouraging sign in the effort to reduce wildlife crime. Through community engagement in income generating activities, more than 40% of encroachment in BBS could be eliminated as the farmer’s profit increased almost 20% on average, compared to their previous agricultural income. At present many farmer groups and villagers from other areas are seeking support from RPU to do the same improvement in their villages.

**Session VIII: In situ Conservation**

**Control of Invasive Arenga Palm (*Arenga obtusifolia*) in Habitat Suitable for Javan Rhino (*Rhinoceros sondaicus*), Ujung Kulon National Park, Indonesia**

Sectionov Inov
International Rhino Foundation Indonesia Liaison

The Javan Rhino (*Rhinoceros sondaicus*) is Critically Endangered according to the IUCN Red List of Threatened Species (http://www.iucnredlist.org/apps/redlist/details/19495/0). Its population is estimated at 35-44 animals confined to Indonesia’s Ujung Kulon National Park, located on the western tip of Java (IUCN 2010). Ujung Kulon National Park is a United Nations World Heritage site and the final stronghold for this species, following its recent extirpation in Vietnam. Unfortunately, suitable Javan rhino habitat within the national park is limited by the spread of an invasive palm (*Arenga obtusifolia*) that threatens to dominate the Ujung Kulon area.

The distribution of *Arenga obtusifolia* in Ujung Kulon renders a significant portion of the tropical forest habitat unsuitable for Javan rhinos. The palm contains sodium oxalate which helps defend it against herbivores such as rhinos, wild cattle and deer. Also, where arenga palm dominates, the growth of other plant species — including many Javan rhino food plants — is suppressed. Currently, an estimated 18,000 hectares (approximately 60% of the park’s land area) is covered by the invasive palm (MoF, 2007). Putro (1997a) described the regenerative ability of arenga palm as high; the tree can regenerate via its roots as well as by seed/fruit dispersal.

The multiple aims of current wildlife research and conservation efforts are to: 1) prevent any increase/reduce the distribution of *Arenga obtusifolia* within Ujung Kulon National Park; 2) increase natural feeding grounds commonly used by Javan rhinos; 3) document Javan rhino habitat utilization pre-and post-clearing of palms on experimental plots; and 4) evaluate the most cost-effective and environmentally-responsible techniques for habitat restoration.

Results obtained to date indicate that the dominant factors affecting palm clearance and regrowth patterns are seasonal weather patterns, light intensity and...
The Role of Standing Sedation in Mitigating Human-elephant Conflict in Sri Lanka

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With the introduction of the commercial dart in 1953, chemical immobilization of wildlife including elephants became a routine management practice. It was in 1967 that the staff of the Department of Wildlife Conservation (DWC) in Sri Lanka was first introduced to the use of the drug M-99 as a means of anaesthetizing elephants by Gray & Nettasinghe (1970). However, such immobilization has its own risks where the elephant can injure itself or die while being anaesthetized. By contrast, standing sedation using xylazine is safer for the elephant and the effect can last longer and be utilized more often than anesthesia. The home-made collar was fastened with just a padlock and chain instead of nuts and bolts for easier and quicker attachment to the elephant being anaesthetized. By contrast, standing sedation using xylazine is safer for the elephant and the effect can last longer and be utilized more often than anesthesia. The home-made collar was fastened with just a padlock and chain instead of nuts and bolts for easier and quicker attachment to the elephant tranquilized under standing sedation. The transmitting GPS/GSM unit comprised of 1.6 kg 100 Ah rechargeable battery to signal the location of the elephant once every four minutes. This allowed us to monitor the elephant online in real-time. The software used is quite versatile to establish geo-fencing where e-mail or SMS alerts could be sent to mobile phones. Thus immediate action is possible to chase the elephant back into the forest before any catastrophe occurs. The software also has the capability to monitor remotely the battery level. As the battery is rechargeable, the elephant could be brought under standing sedation to replace the old collar and replace it with a new one for continuous monitoring. Online monitoring also reveals daily behavioral patterns such as patterns of utilization of habitats, the number of attempts the animal makes to raids crops and fine-tuned movement patterns including resting times and the distance traveled each day.

Which Future for Human-Elephant Coexistence in the Boucle du Mouhoun Region (Burkina Faso)?

Julien Marchais
Des Éléphants & des Hommes

In 2002, an aerial count of the elephants of the Boucle du Mouhoun Region, in Burkina Faso, was conducted. The methodology used was a sampling count and it gave the following results: 99 direct observations and an estimate of 541 individuals (+/- 320 - 95% CI). Since then, specialists have discussed these figures and many have thought that the real elephant population was probably closer to the lower limit, including the author of the 2002 count himself.

In 2006, our NGO “Des Éléphants & des Hommes” (Elephants & Humans) started a program in this region. The situation was very difficult as most of the elephants’ natural habitat had deteriorated and the protected areas were poorly equipped to be efficiently controlled. The presence of cattle was the main pressure on the wildlife habitat. Illegal wood collection, poaching for small and middle sized mammals, uncontrolled fires and land encroachment for agriculture were the other main pressures. The elephants were very difficult to see in the whole of their Mouhoun range, except in the Deux-Balé National Park, the most protected area of the complex. There, elephants were fairly easily observed during the dry season. We started working in this National Park hoping to contribute to the beginning of a rehabilitation process. From 2007 to 2009, we initiated an environmental education program with the main objective of offering a chance for primary school students and their teachers to see their elephant neighbors and natural heritage. It is interesting to note that over 80% of the students had never seen an elephant before!

In 2010, as we hoped, a rehabilitation process was initiated by the government so we adjusted our program to encourage and enable the process. From 2010 to 2013, in partnership with the various stakeholders, we continued the educational program and extended it to 250 primary schools. We also organized over 20 training sessions to develop alternative income generating activities for the communities compatible with the preservation of the elephants and their habitats, we equipped over 150 producers, we offered training and equipment to reduce and avoid human-elephant conflict on the fringe of the Deux-Balé National Park, we supported ranger patrols and provided equipment. We also conducted studies and research.

The idea is to secure Deux-Balé National Park, where elephants will be free to quietly live, and contribute to the prosperity of its periphery so that the human inhabitants can fully benefit from the rehabilitated national park. We are only at the beginning of the process and it is far too early to know if the project will be successful. But time is running out and pressures remain on the other wildlife areas of the complex. Pressures also still remain on the Deux-Balé National Park due to the destructive activities of the neighboring communities. The question is then: are we winning? Is it still possible to win? In order to have part of the answer, in 2013 we conducted a new aerial survey of the elephant population. It was led by Dr. Philippe Bouché and the National Office for Protected Areas of Burkina Faso, this time using a total count methodology. The results are frightening...

This project funded by the International Elephant Foundation and the Amnéville Zoo.
The Conservationists’ Dilemma: A Need for Pragmastism Regarding Captive Breeding of Exotic Wildlife


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Over this past century, the primary mission of zoos has evolved from collection and exhibition to education and conservation. Strategies for the latter have included captive breeding programs in attempts to protect and expand gene pools of species threatened by inexorable losses of natural habitats and, for rhinoceroses and elephants, by widespread escalation of criminal poaching stimulated by increasing demands for rhinoceros horn and ivory to supply Asian black markets (1).

Advocates for captive breeding of rare species emphasize some secondary benefits: opportunities for more extensive, controlled physiological studies, and frequently lethal anemias, vasculopathies, dermatopathies, neural developmental abnormalities, and iron storage disease (ISD).

Iron overloading was first detected in captive African black rhinoceroses by Smith et al. (3), and subsequently found to affect other perissodactyls, Sumatran rhinoceroses (4–6) and tapirs (7), as well as numerous other species of exotic wildlife (8). There is strong evidence that excess iron causes or contributes to many of the aforementioned disorders acquired by browser rhinoceroses under duress of unnatural diets and altered social and environmental conditions (4, 5, 9).

All rhinoceroses are inherently impaired in their capacities to neutralize ambient oxidants (10–12), so they are highly vulnerable to the toxic effects of hydroxyl and other free radicals that are catalytically generated by elemental iron.

While we have focused on rhinoceroses, it is important to emphasize that progressive iron overburdens develop in many mammalian, avian, marine and other species when extracted from their native environments. ISD therefore represents a global anthropogenic challenge to conservation medicine. Cost/benefit analyses of in situ vs. ex situ conservation programs are now further complicated by this philosophical consideration: Is it ethically justifiable or pragmatically possible to preserve certain wildlife species by dislocating them from their natural habitats even if captive conditions are known unequivocally to induce pathological disorders of high morbidity and mortality? This paradox should be thoughtfully considered along with potential alternatives such as in situ sanctuaries and intensive protection zones (13, 14) in range countries where availability of natural forage and environmental/social conditions might prevent or ameliorate captivity-induced disorders and dyscrasias.

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Trend Analysis of Temporal and Spatial Patterns of Human-Elephant Conflict in Nepal
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This study addresses spatial and temporal patterns of Human Elephant Conflict (HEC) in Nepal. This study is a compilation of available historical records published electronically in 8 daily national newspapers over a 10 year period (2003-2012) of elephant attacks on humans and property and resultant elephant mortality. In addition to direct losses, current mitigation measures are inadequate due to limited resources and are not data-driven. Over the past decade, HEC has caused 103 human deaths, 52 serious human injuries, and 642 cases of extensive property damage; additionally, there have been 18 elephant deaths and 6 severe elephant injuries. Data were analyzed using ANOVA, regression, $\chi^2$ test, correlation, and trend analysis to investigate temporal and spatial patterns of conflict. HEC intensity was high in the migratory route along the Indo-Nepal border region. There was a high correlation between elephant and human casualties ($\rho = 0.802, p < 0.001$). Seasonal variation in HEC was significantly different ($\chi^2 = 117.219, p < 0.001$) with greatest conflict occurring during the winter associated with rice harvest. Other seasons with elevated HEC include summer, associated with the harvest of maize, and late winter/early spring, associated with the harvest of wheat. HEC has significantly increased in the number of incidents in more recent years ($\rho = 0.905, p < 0.001$). Most human casualties occurred during the rice harvest period, and were mature males with an age range of 40-70 years. Most elephant casualties occurred during the maize harvest period. As elephant invasion is greatest during harvest of several crops, proper land use planning, and promotion of alternative cropping patterns should be considered for the mitigation of HEC.

Session IX: In situ Management of Wildlife and Habitat

The Significance of Pre-Existing Social Bonds in Translocated Black Rhinos
Natasha Anderson
Lowveld Rhino Trust

Black rhinos (Diceros bicornis) are generally assumed to be solitary animals apart from cow-calf pairs and transient mating combinations. This perception induces some rhino management suggestions that may not adequately take rhino social factors into account. For instance, a standard recommendation in rhino metapopulation management is that to counter the loss of genetic diversity through genetic drift, at least one new founder should be introduced into a sub-population every generation. This recommendation implies that a rhino can be taken from one population to another without major social problems. Experiences in translocation operations that the Lowveld Rhino Trust has been involved in suggest that pre-existing social bonds between rhinos are more significant to the success of such operations than has generally been appreciated.

Between 2003 and 2010, 121 black rhinos were introduced into a 1,000 sq mile section of Bubye Valley Conservancy (BVC), Zimbabwe. These translocations have provided a unique opportunity to observe black rhino behaviour as they involved the phased relocation of entire social groups. The translocations were undertaken in response to an expansion, year by year, of human settlement and of poaching pressure within Bubiana Conservancy. The rhino populations in both areas were monitored at the individual level, so associations between individual rhinos were known both before and after the series of translocations. Black rhinos occupy fairly stable home ranges, with dominant bulls overlapping their ranges with those of various cows and sub-dominant animals. Hence rhinos that tend to associate together can be regarded as neighbours.

Not all neighbours were translocated from the one conservancy to the other in the same year, because logistical and political constraints limited the scale of each annual operation. Nor were all rhinos released from the same point in BVC. Nonetheless, a clear tendency was shown by rhinos to re-associate with the same neighbours that they had in Bubiana Conservancy, despite these re-associations requiring some rhinos to move significant distances through unfamiliar territory.

Other observations have been made of rhinos that struggled to fit into the BVC population after being brought in as complete strangers from other populations. Further indications of the significance of pre-existing social bonds in translocation success are shown by the outcome of long-distance translocations of black rhinos from South Africa to North Luangwa National Park in Zambia.

The conclusion from these translocation experiences is that more tranquil re-introduction scenarios (i.e. less dispersion, less fighting) can be achieved by restocking with rhinos that already know each other prior to their translocation.
Boma Adaptation and Development of a Scoring System for Recently Captured White Rhinoceroses (*Ceratotherium Simum*) In South Africa

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One hundred nine sub-adult and adult white rhinoceroses (*Ceratotherium simum*) were captured between 2009 and 2012 in Kruger National Park and placed in holding bomas prior to translocation to other locations within South Africa. Parameters associated with immobilization, physiological, nutritional and other measurements were recorded at the time of capture and compared between rhinoceroses that adapted to boma conditions and those that were maladapted to determine if there were any predisposing factors. A simple four category system was developed to assess boma adaptation, based on appetite, demeanor, defecation, and activity. Individual and total median scores were used to determine trends and when the animal had successfully adapted to the boma. Twenty-one rhinoceroses did not adapt to the boma conditions and were released. Physiological and nutritional measurements were measured at the time of release from the bomas and compared in individual rhinoceroses between the time of capture and release as well as between adapted and maladapted groups.

Based on these criteria, 19.3% (21) of rhinoceroses were maladapted with an additional 5.5% (6) having some minor complication which did not require early release. Adapted animals had a mean length of boma confinement of 89.9 days with a range of 39-187 days, while maladapted rhinoceroses had a mean length of boma confinement of 13 days with a range of 8-16 days. The second week in the bomas was a critical period in which statistically significant differences in boma scores were observed between adapted and maladapted groups. Rhinoceroses which did not reach a threshold score by the end of the second week or showed a decline in score during this period required early release.

There were no differences in gender or age groupings between adapted and maladapted rhinoceroses. Differences in immobilization protocols, physiological and other capture-related measurements did not appear to be correlated with whether animals adapted to boma conditions. Evaluation of hematologic, biochemical, mineral, and vitamin panels at the time of capture showed no clinically significant differences that could be associated with boma adaptation. However, boma-adapted rhinoceroses did show a few changes between capture and release in values indicating decreased feed intake resulting in mild-moderate negative energy balance. Blood from boma-maladapted rhinoceros demonstrated stress leukocytosis at the time of release. Biochemical values suggested catabolic states due to negative energy balance and was consistent with the large mean weight loss in rhinoceros despite the short time in confinement.

In conclusion, using the scoring system to assess key measures of boma adaptation, a characteristic pattern was documented in rhinoceroses that did not adapt. These patterns became distinct between adapted and maladapted animals by day 8 and typically resulted in release of individuals by day 16. There were significant changes in maladapted rhinoceroses that would likely lead to serious consequences if early and rapid intervention was not undertaken. Application of this newly developed boma scoring system will be a useful tool for scientifically-based decisions for intervention in boma management of white rhinoceros and has potential uses for other species.

ACKNOWLEDGEMENTS

The work was supported by a grant from the International Rhino Foundation and South African National Parks. The authors wish to thank the entire staff of the Veterinary Wildlife Services team for their support in this project.

Contact Calls of the Northern and Southern White Rhinoceroses: Source of Information on Individual Identity and Species of the Caller?

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Communication of the northern (*Ceratotherium cottoni*) and southern white rhinoceros (*Ceratotherium simum*) has been until now studied only very little. The rhinos have poor eyesight and vocal and olfactory signals are the most important for their communication, however, what information are the rhinos able to transmit and perceive remains unknown. Vocal repertoire of some rhinoceros species has lately been described, nevertheless, studies reporting any information encoded in their calls are completely missing. While rhinos are the most social from all rhino species; well-developed communication system might therefore be especially useful to them. We studied contact call ‘pant’ of the northern and southern white rhinos, which is formed by a series of inhalations and exhalations and which does not have parallel in any other rhino species. We investigated if pant calls contain information about individual identity and species of the caller. Such ability, in addition to olfactory cues, would allow rhinos to communicate with highly increased accuracy. We recorded and analysed 385 pant calls of six northern and 14 southern white rhinoceroses in several zoological gardens and South African wildlife reserves. Discriminant analysis assigned 86% (77% cross-validated) of pant calls to correct individual, which is significantly more than would be expected by a chance. The most important parameters for distinction between individuals were temporal parameters such as duration of the longest inhalation in call or call duration and ratio of harmonic to nonharmonic energy in an inhalation. Calls of individuals clustered into apparently separated groups according to the species and both species significantly differed in call duration and some frequency parameters of their calls. Our results also suggest the influence of age class and social status on the call structure of males. White rhino pant calls have complex structure and can potentially encode also other information. They might thus represent more sophisticated communication system so far unknown in rhinos. Better knowledge of vocal communication of northern and southern white rhinoceroses might be extremely valuable for improving their management in zoological gardens and wildlife reserves.
The Vanishing Asian Elephant Corridor in the Brahmaputra Valley, Assam: A Threat to Asian Elephant Conservation

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The habitat loss and degradation has been widely recognized as the primary threat to the survival of the endangered (IUCN) and schedule-I (WPA, 1972) Asian elephant (*Elephas maximus*) as the habitat loss directly depresses population size. A problem in dealing with the above threat arises from the fact that, maintaining sufficient contiguous elephant habitat is difficult. The development activities like widening and construction of roads, highways, railway lines on the southern and northern bank of the river Brahmaputra were responsible for fragmentation of the fairly contiguous forest and destruction of corridor responsible for hampering gene flow on the both northern and southern bank of river the Brahmaputra. The river Brahmaputra, National Highways, railway lines may sometimes act as the landscape barrier for gene flow.

Hence, this has been found very much necessary to evaluate the Asian elephant corridor present in both the northern and southern banks of the river Brahmaputra. The main objective of the present study was to study the corridor utilization frequency by elephants in the Brahmaputra valley, Assam with corridor distribution mapping in the valley. Study was done during the year 2012-2013 in the Brahmaputra valley, Assam.

Belt transect method was used to assess the dung density of Asian elephant in the corridor to assess the frequency of utilization of the corridors during the period of the study. DGPS readings of the corridors were recorded with its extent in the forest and non forested area. GIS map were prepared on the distribution and extent of the Asian elephant corridor in the Brahmaputra valley by ArcGIS 10 software using the LISS III, FCC image.

During the present study it has been found that, there have been nine Major Elephant Ranges found in the State of Assam (ERs) covering the elephant bearing areas. In the entire the Brahmaputra valley, there were 17 corridors were which were used frequently by the Asian elephant. Amongst the corridors, the Asian elephant used the Daodhara-Bornadi very frequently with relative percentage of use 9.77 %, which was followed by Bornadi-Kholingduar (8.66 %), Kaziranga-Burhapahar (8.32 %), Kaziranga-Panbari (8.14 %), Kukurakata-Bagser (7.7 %), Kalapahar-Doigrung (7.24 %), Kotha-Burhidihing (6.75 %), Ripu-Chiranj (6.37 %), Nambor East-Nambor West (5.89 %), Manas-Gabharukhunda (Bhutan) (5.34 %), Dulung-Subansiri (4.87 %), Uperidihing east-Uperidihing West block at Bogapani (4.39 %), Sankosh-Jamduar (4.37 %), Uperidihing east-Uperidihing West block at Golai (3.99 %), Laming-Arrang RF (3.71 %).

These corridors in the Brahmaputra valley have been found very much important in maintaining the Asian elephant gene flow between different population in both north and south bank of the river Brahmaputra. Current conservation and management strategies have increasingly being designed at the landscape scale, wherein a network of interconnected habitat patches can together support healthy populations, where the role of corridor is very much important. Such an approach can reverse the effects of habitat fragmentation, and can greatly reduce extinction threats faced by an isolated population. Hence, the Asian elephant corridors in the Brahmaputra valley, Assam should be notified with permanent demarcation on ground. All the activities such as developmental activities causing interruption to elephant movement, road construction etc. should be stopped permanently for the conservation of Asian elephant corridor.

Human - Elephant Conflict in the North West Wildlife Zone of Sri Lanka

Pubudu Darshana Weeraratna
Species Conservation Centre, Sri Lanka

Sri Lanka has the second largest wild Asian elephant population and the highest wild elephant density among the 13 range countries. The estimated current population of wild elephants according to the census conducted in August 2011 is around 5879 (Dissanayaka, DWC 2011). According to the Department of Wildlife Conservation (DWC), 553 humans and 1409 elephants have died during the past eight years (2005-2012) due to the conflict (Figure 1). In the year 2011 alone 255 elephants were killed and the main reason of those deaths was gunshot injuries. In the same year 60 people were killed by wild elephants (DWC, 2012).

The North Western Province comprising of two administrative districts, Puttalam (3,013 km2) and Kurunegala (4,813 km2) supports nearly 20% (1189 elephants) of the estimated elephant population of Sri Lanka (DWC, 2011). This elephant population is scattered in small pockets of habitats throughout the NWP as herds and individuals. Highest number of elephant and human deaths were recorded from the North Western region, the most affected area in the county. Location and the number of elephants in two Districts and 14 Divisional Secretariats were identified in conflicted areas of the North West wildlife zone. When analyzing the data of Department of Wildlife Conservation (DWC) for the past eight years (2005-2012); 226 humans and 556 elephants have died in this region (DWC 2012).

Large areas of land have been cleared and extensively planted with crops that are palatable for elephants such as Banana and Paddy. The main objective of the research was to identify human elephant conflict accelerating causes; and identifying the exact locations for development projects, that do not block the natural trails and also that do not harm the natural food sources of the elephants. The next concern was promoting cultivation of crops, changing the possibility of the periods and make.

Elephants are killed due to various reasons. The main reason is human-elephant conflict where elephants are simply killed because they interfere with agriculture and the lives of the villagers. Some of the major causes of elephant mortality include injuries sustained due to gun shot or trap guns, electrocution, poisoning, land mines, accidentally falling into wells and abandoned sand pits, collision with trains, trucks and natural causes. Poaching for ivory though rare is now carried out under the disguise of the conflict and continues to contribute to eliminating the few remaining tuskers.
Managing the conflict is the key to effective elephant management in Sri Lanka. DWC is capable of protecting the elephants within the protected area network, but acknowledges that ensuring the long-term survival of the elephant population outside the protected area network, though difficult, is critical for their long term survival.

Session X: Reproduction II

GnRH Vaccination as a Treatment for Reproductive Tract Pathologies in Female Elephants (Contraception for Post-Reproductive Cows? Why Close the Door After the Cow is Out?)
Nancy Boedeker, Dennis Schmitt, and Janine Brown
Smithsonian’s National Zoological Park

As the populations of Asian and African elephants in zoos ages, many females develop ovarian and uterine pathologies, including cystic endometrial hyperplasia and leiomyomas. Though often benign, these lesions can be associated with a bloody discharge, colic, anemia and decreased fertility. It has been suggested that the excessive number of reproductive cycles and steroid hormone exposure experienced by elephants in the captive population is an important factor in the development of these pathologies (“asymmetrical reproductive aging”). Wild elephants, by comparison, generally cycle far fewer times in their lives since they are often pregnant or lactating. We hypothesized that cessation of ovarian cyclicity would decrease the development of reproductive tract pathologies and minimize their negative effects by decreasing uterine size and vascularity. GnRH vaccines have been designed to stimulate the production of anti-GnRH antibodies that block the binding of endogenous GnRH to gonadotrope receptors in the pituitary gland. This action inhibits the release of FSH and LH from the anterior pituitary, thereby causing the cessation of ovarian steroidogenic activity and reproductive cyclicity. Our hypothesis was supported by the effectiveness of a GnRH vaccine (Repro-BLOC, Amplicon Vaccine, LLC, Pullman, WA) to suppress ovarian cycle activity and resolve hemorrhage and anemia associated with a suspected vascular reproductive tract tumor in a 59-year-old Asian elephant (Elephas maximus). Six years after initial vaccination, this elephant continues to lack distinct ovarian cycles and is healthy. Because Repro Bloc is not commercially available in the U.S., we initiated a larger study to evaluate the efficacy of Improvest, a GnRH vaccine produced by Pfizer, Inc. (New York, NY) to suppress ovarian cyclicity in non-breeding female elephants that have been diagnosed with reproductive tract pathologies by rectal ultrasound. The goal is to identify an appropriate vaccination protocol to resolve reproductive pathologies in female elephants and determine duration of effect and reversibility. This will be accomplished by monitoring of weekly serum hormone levels (progesterone, FSH, and LH) and GnRH antibody titers, the pituitary response to a GnRH (Cystorelin) challenge, and a follow-up ultrasound. A total of 7 elephants are at varying stages of the vaccination study protocol, but preliminary hormonal and antibody response data are promising. In addition to resolving reproductive tract problems in older females, there is also interest in the use of GnRH vaccination for contraception to minimize human-elephant conflicts and competition for resources in areas where suitable elephant habitat to sustain large populations is limited.


Oral Imipramine and Intravenous Xylazine for Pharmacologically-induced Ex Copula Ejaculation in an African Elephant (Loxodonta africana)
Ray L Ball, DVM and Chris Massaro
Tampa’s Lowry Park Zoo

Inducing ejaculation ex copula as method for semen collection is a standard approach for semen collection in managed elephants. The current methodologies involved rectal massage to induce ejaculation. While some success has been achieved and successful inseminations have resulted, there is tremendous effort in this and room for improvement. Domestic equids have had pharmacologically-induced ex copula ejaculation with various drugs for decades with varying success. Current recommendations are to incorporate the anti-depressant imipramine at 3mg/kg orally followed in two hours by the alpha-2 agonist xylazine 0.66mg/Kg intravenously to facilitate semen collection in equids. An ejaculate is typically produced from 3-20 minutes. This approach was applied to a 23 year old, 4223kg, male African elephant to facilitate an artificial insemination attempt for a multi-parous 28 year old female that was behaviorally incompatible with him.
A trial dose of 12500mg imipramine (mg/kg) po was followed by 100mg xylazine (0.024mg/kg) IV two hours later. There was a very light tranquilization with his penis protruded but after 20 minutes the elephant moved out of the chute and recovered uneventfully. Later a few drops of fluid were reported to be seen dripping from his penis but these were not recovered.

Over the next 6 days, 4 additional trials were made with imipramine ranging from 12500 to 20500 (2.95 – 4.85mg/kg) followed by 170mg xylazine two hours later IV. Sedation was greatly improved and various amounts of fluid and bellowing were exhibited in each session except the final when the initial dose of imipramine was not delivered completely. On the 3rd attempt the bull was given 20500mg imipramine PO and then two hours later 170mg xylazine IV. Over the next half hour no fluid produced but approximate 30 minutes late the staff note a clear fluid from him. This sample had some sperm in it and good motility. The bull was moved back to ERD and a very light rectal massage produced several fractions of ejaculate. The first couple of fractions was clear to slightly cloudy and had a total volume of about 3ml. Little sperm was seen in these samples. The next three tubes were thick white/creamy fluid with a thick coagulum. A total volume of about 4ml of sperm rich fluid was recovered with essentially all alive. They were moving into the coagulum seen, but when diluted with equal volume of Heps they essentially all (90% or better) had forward motility. This sample was diluted with 12ml of Hepes and utilized for an insemination attempt within 3 hours.

A combination of imipramine followed with xylazine and a light rectal massage may hold some potential for enhanced semen collection in elephants. Samples may be sperm rich without fluid from accessory sex organs as is reported in horses.

Suppression of Testicular Function by Means of a GnRH Vaccine in African Elephant Bulls
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Introduction
In this study on 13 African elephant bulls, we examined the effect of the commercial piglet GnRH vaccine Improvac® (Pfizer Animal Health) on reproductive organs, hormone levels and sperm production. Aim was to test, if this vaccine is a potential measure for aggression (musth) and fertility control.

Over a two year period, 2 wild and 11 captive African elephant bulls in South Africa were examined every six months to record the changes. After first vaccination and an initial booster 6 weeks later, each bull received a booster vaccination every 5-6 months, followed by a full examination 4 weeks later. Each bull was injected with 5 ml of Improvac® deeply intramuscular into the gluteal muscle during each vaccination, either by hand injection or dart. Table 1 gives an overview of the males examined.

The examinations took place while the bulls were either fully immobilized (M99/Azaparone) or in a standing sedation (Medetomidin OR Detomidin/Butorphanol) within a chute or pen system (Fig. 1). Blood samples and body measurements were taken, a transrectal ultrasound and a semen collection (either by electro ejaculation or manual prostate massage) were performed.

![Fig. 1 semen collection by transrectal massage or by electro ejaculation in lateral recumbency.](image-url)
Results

During the 2-year study course, each individual received 6 GnRH injections. Serum testosterone levels dropped to non-detectable levels after the second injection in 6/13 bulls and after the 3rd injection in the remainder of the bulls. Similar, at the second examination (6 months after start of treatment), the testicle diameter had already significantly declined. At the end of the study, the testicle size (measured as the area in the 2D ultrasound image) had declined by almost 60% on average, showing the dramatic effect of lacking GnRH stimulation (Table 1).

While the semen collection showed viable spermatozoa in all but two bulls prior to GnRH vaccination (bull#3 was still premature and bull #2 was treated with the vaccine when he was younger), after 3 vaccinations, either no sperm were found anymore or immotile spermatozoa with a large proportion of head and tail separation were detected (Fig. 2). Seminal plasma was still produced, however the volumes also declined. This was reflected by the reduced size and fluid content of the accessory sex glands (seminal vesicles and ampullae) as seen in the ultrasound image.

None of the bulls came into musth, and in the oldest bull of the study (bull #12), who was in musth when he received his first injection, musth ceased immediately and was not observed again.

<table>
<thead>
<tr>
<th>#</th>
<th>Elephant Name</th>
<th>Age (years)</th>
<th>Status</th>
<th>Height (cm)</th>
<th>Testicle size (area) prior to treatment (cm²)</th>
<th>Testicle size (area) at end of study (cm²)</th>
<th>Testosterone level prior to treatment (nmol/l)</th>
<th>Testosterone level at end of study (nmol/l)</th>
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<tr>
<td>1</td>
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<td>22</td>
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<td>281</td>
<td>157,7</td>
<td>66,4</td>
<td>20,5</td>
<td>0,0</td>
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<tr>
<td>2</td>
<td>Mooketsi</td>
<td>19</td>
<td>captive</td>
<td>245</td>
<td>175,4</td>
<td>35,7</td>
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<td>0,0</td>
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<tr>
<td>3</td>
<td>Nduna</td>
<td>8</td>
<td>captive</td>
<td>212</td>
<td>68,9</td>
<td>46,8</td>
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</tr>
<tr>
<td>4</td>
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<td>10</td>
<td>captive/wild</td>
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<td>22,0</td>
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<tr>
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<tr>
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<td>58,5</td>
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</tr>
<tr>
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<td>17</td>
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<td>4,2</td>
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<tr>
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<td>58,7</td>
<td>46,8</td>
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<tr>
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<td>269,2</td>
<td>157,9</td>
<td>63,4</td>
<td>22,4</td>
<td>0,0</td>
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</tr>
</tbody>
</table>

Table 1. Overview of animals examined, showing the sharp decline in testicle size and serum testosterone concentration.

In some animals, behavioral changes, mostly in connection with dominance behaviors amongst other bulls, were observed. Examples include less tension between certain bulls, or mounting of treated bulls by untreated bulls.

In two other cases, bulls that were known to dismantle gates or fences, were not observed to break anything ever since the vaccinations started. However, the general character of the bulls remained and, besides two cases were handlers found their free contact animals more submissive, no changes of behavior towards the keepers were observed. In one case, a bull was even switched from free contact to protected contact during the vaccination trial.

Fig. 2 Typical image of head and tail separation in the ejaculates after 3 vaccinations.
Discussion

So far, our methods have been effective to study the consequences of the GnRH vaccine in elephant bulls. In all animals studied, we have seen a dramatic decrease in testicle size, testosterone level and sperm quality. Therefore, it appears save to assume that the GnRH vaccine is effective as a contraceptive or does at least greatly diminish fertility in elephant bulls.

Although testosterone was suppressed and no musth phases occurred, there were only minor behavioral changes in certain bulls. This shows that by large parts, the character of each animal and the learned behaviors play a more decisive role than the hormone status.

We plan to further monitor these elephants, as we need to look into long term effects as well as how long effects last. The following objectives are still to be tested:
- Long term effects
- Reversibility
- Application in a breeding herd to ultimately proof sterility of vaccinated bulls
- Long term monitoring of behavior

By taking certain elephants off the vaccine, we would gather information on the length of vaccine intervals and time till effects reversed. Currently, we administer the vaccine every six months. But it is probably safe to apply it only once a year after intensive initial treatment.

Acknowledgement

We wish to thank the International Elephant Foundation and the U.S. Fish and Wildlife Service, African elephant fund for funding the research in South Africa and the Birmingham zoo for supporting the hormone analysis.

Pretreatment of Asian Elephant (Elephas maximus) Spermatozoa with Cholesterol-loaded Cyclodextrins and Glycerol Addition at 4°C Improves Cryosurvival

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Asian elephant spermatozoa are sensitive to chilling and do not respond well to cryopreservation. The objectives of the present study were to: (1) determine whether cholesterol content can be modified by preincubation of Asian elephant spermatozoa with cholesterol-loaded cyclodextrin (CLC); and (2) assess the effects of CLC concentration(s), temperature at time of glycerol addition (22°C vs 4°C) and dilution medium on post-thaw sperm survival. Spermatozoa incubated with 3.15 mg CLC exhibited increased (P < 0.05) cholesterol concentrations. Pretreatment of spermatozoa with 1.5 mg CLC resulted in improvements (P < 0.05) in all post-thaw parameters. Glycerol addition at 4°C also improved all post-thaw parameters compared with 22°C. Dilution of thawed spermatozoa in an egg yolk-based medium improved (P < 0.05) motility compared with Ham's F-10 culture medium. In summary, our findings indicate that modifying cholesterol content within the plasma membrane improves the cryosurvival of Asian elephant spermatozoa. The development of an improved cryopreservation method that includes modification of membrane cholesterol and the addition of glycerol at 4°C, as reported in the present study, is an important step towards utilization of cryopreserved spermatozoa in captive management of this species.

Successful Cryopreservation of Asian Elephant (Elephas maximus) Semen Using Simple Low-tech Techniques.

Danielle Arnolda, Charlie Grayb, Terri L Rothic and Laura H. Graham

U.S. Fish and Wildlife Service, African elephant fund for funding the research in South Africa

Asian elephant populations are under increasing threats in the wild and the rate of reproduction in zoos is not consistent enough to sustain a genetically diverse population to act as a hedge against extinction. The use of assisted reproductive technologies like artificial insemination combined with cryopreservation of gametes and embryos could be a valuable tool for the conservation of elephants. However, simple low-tech protocols for the cryopreservation of Asian elephant semen are lacking. The purpose of this study was to compare the effect of simple freezing techniques and different thaw temperatures on the post-thaw quality of cryopreserved Asian elephant semen samples. Ejaculates (N = 3) were collected by rectal massage from two bulls. The average initial motility was 75.6% (range 72 – 80%) and the average proportion of intact acrosomes was 76.3% (range 54 – 90%). Semen samples were immediately extended in a lactose/egg-yolk-based extender (BC solution) and then cooled to 4C in an equilibration over 3 hrs. Once cooled, samples were further extended 1:1 with glycerolated extender (14% glycerol for a final concentration of 7%) and the average proportion of intact acrosomes was 76.3% (range 54 – 90%). Semen samples were then loaded into 0.5 ml straws. Semen samples were cryopreserved by placing straws in LN2 vapour at 5 cm, 2 cm and 1 cm above LN2 as well as by placing straws directly into a charged LN2 dry shipper. After storage in LN2 for at least 48 hrs, straws were thawed at 37°C for 30s, 50C for 10s, 50C for 15s or 75C for 6s and diluted 1:4 in non-glycerolated extender. Samples were assessed after cooling (pre-freeze), immediately post-thaw and after incubating thawed samples at 37C for 1 hr. Cooling had no significant effect on acrosomal integrity or motility (P>0.05). Only the ejaculate that initially had a low proportion of intact acrosomes lost motility with cooling (initial motility of 72% decreased to 59%) and it had the poorest preservation of pre-freeze motility in all treatment groups. Semen samples cryopreserved by placing straws at 2 cm and 1 cm above LN2
or placed directly in the dry shipper had significantly higher post-thaw motility and intact acrosomes than straws placed at 5 cm above LN2 (P < 0.001). Thaw temperature had no significant effect on post-thaw motility or acrosomal status (P>0.05). Placing straws directly in the dry shipper and thawing them at 75C consistently preserved >60% of the pre-freeze motility (average 82.5%; range 61 – 100%) and acrosomal status (average 87.5%; range 64 – 100%). There was no significant loss of motility observed in the thawed samples after 1hr incubation at 37C. These initial results suggest it is possible to successfully cryopreserve Asian elephant semen using simple low-tech techniques. This study is ongoing with results from additional ejaculates and treatments being assessed and is funded by the International Elephant Foundation.

**Session XI: Ex situ Reproduction and Management**

**Relationships Among Birth Presentation, Amniotic Sac Rupture and Stillbirths in Rhinoceros**

Jane Kennedy

San Diego Zoo Safari Park

International Rhino Keeper Association

In spring 2012, the San Diego Zoo Safari Park experienced a difference between two greater one-horned rhinoceros births. One was a successful live birth, anterior presentation, and the other was a dystocia stillbirth, posterior presentation. Initially the stillbirth was suspected to be due to the posterior presentation at birth. However, years of anecdotal evidence suggested otherwise and a formal investigation was initiated.

Data were gathered by reviewing animal records including behavioral and breeding records for all three rhino species at the San Diego Zoo Safari Park. Data on 173 rhino births between 1970 and 2013 were analyzed for species of rhino, and whether or not the birth was recorded as a live birth or stillbirth. Breeding records indicated that 5.3% (n = 93 total births) of the southern white rhinos born at the park were stillborn compared to 0.6% for black rhinos (n = 15 total births) and 24.5% for greater one-horned rhinos (n = 65 total births). Subsequent analyses of regional studbook records for each of these species through 2010, indicated that stillborn calves account for 7% of southern white rhino, 11.0% of black rhino, and 19.7% of greater one-horned rhino births in North America. This information led to further investigation as to why greater one-horned rhinos have such a high rate of stillborns.

While data are limited, 12 births have been video recorded at the San Diego Zoo Safari Park including 8 greater one-horned, 2 southern white and 2 black rhinos. Seven additional births had written documentation for presentation and are included but are not considered for sac rupture analysis. Of the 19 births, 11 calves were delivered in posterior presentation, 7 were delivered in anterior presentation, and one was unknown. Eight of the 12 births that were recorded were live births, 4 were stillborns. In all four cases of a stillbirth delivery, the amniotic sac ruptured prior to delivery of the stillborn. Three stillborn calves were posterior presentation, and one was unconfirmed. Additionally, the amniotic sac remained intact prior to delivery in all greater one-horned live births recorded. However, one black rhino’s amniotic sac ruptured prior to birth but the calf was still viable, so the number of minutes between sac rupture and delivery may be significant.

With the limited results and surrounding questions, rhino births published on Youtube were then incorporated into the data. The caveat for the addition of these recordings is that all births are live births, as no facility would be expected to post video of a stillbirth. Nine Youtube videos of rhinos giving birth were evaluated; all nine rhinos had the amniotic sac intact at time of delivery.

Results of this study indicate that rhinoceros are able to deliver viable calves presented in both anterior and posterior positions, and that premature amniotic sac rupture may be the more significant factor contributing to the delivery of a stillborn calf.

**Thermoregulation in the African Elephant and Possible Effects on Fertility.**

Ray L Ball, DVM1; Matt James1, M.J. Schotsman2, J.L. Atkinson2, E.J. Finegan2 and S.P. Miller2

1Tampa’s Lowry Park Zoo, 2University of Guelph, Dept. of Animal Sciences

As large mammals in hot, arid environments, African elephants may face difficulties losing excess heat gained during the day. They may store heat gained during the day, and release it to the cool night sky through vasodilation in their ears. Three captive African elephants were observed over six nights and their core body, side, and ear temperatures were measured with thermal imaging. Ear and side temperatures decreased faster on cold nights than warm nights. Both ear and side temperatures were more variable on warm nights. Core body temperatures varied by as much as 4 degrees C as measured by thermal imaging of freshly voided urine. Benedict and Lee (1936) showed a strong correlation between core body temperature and urine temperature, it can be assumed that core body temperature is undergoing these same fluctuations. These findings indicate adaptive heterothermy where heat is stored during daylight hours and subsequently lost overnight. Studies in semi-free-ranging African elephants have shown a very mild core temperature fluctuation as measured by differing methods. Data loggers were given to elephants and were retrieved from the faeces. Mean body temperature over the July 2006-November 2007 study period was about 36.4 +/- 0.03 degrees C and mean daily amplitude was about 1.2 +/- 0.04 degrees C (Hidden, 2009).

Landscape use (Kinhart 2007) and perhaps even captive diets, with highly fermentable concentrates, may contribute to the measured differences between captive and free-ranging elephants, even given differing methodologies. The measured rises in temperature in captive elephant’s raise some questions about possible contributions to infertility in captivity. Heat stress is a well-known cause of both male and female infertility in various livestock
species, mice, and humans. Heat stress has been shown to alter the duration of estrus, colostrum quality, conception rate, uterine function, endocrine status, follicular growth and development, luteolytic mechanisms, early embryonic development, and fetal growth (Jordan 2003). Heat in males will contribute to oxidative stress and decrease in fertility (Tremellen 2008). Environmental factors such as shade, indoor housing overnight, and obesity may contribute to the differences seen between wild and captive elephants in terms of thermoregulation and fertility although the total number of animals both in the wild and captive studies is tantalizingly small. Retrospective analysis of the fertility of the previously studied elephants as well as prospective studies paring thermal imaging with assessments of fertility may prove useful.

Hidden, Philippa Ann, Thermoregulation in African elephants (Loxodonta africana), MSc. University of the Witwatersrand, 2009

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With fewer than 5000 black rhinoceros (Diceros bicornis) left in the wild, ex situ populations play a vital role in the conservation of this species. However, the European captive population of the eastern subspecies (D. b. michaeli) is currently underperforming compared to their in situ counterparts, with annual growth rates of only 1-2%, compared to >5% in situ. In recent years, the primary factor limiting growth of this population has been low rates of reproduction, with only around 11% of adult females breeding each year. Furthermore, approximately 40% of reproductive-age individuals are yet to successfully produce offspring, resulting in high reproductive skew in both males and females. To investigate differences in reproductive success, faecal samples were collected from 23 males and 39 females at 13 institutions across Europe, and used to measure reproductive and adrenal hormones. In females, three-quarters of all oestrus cycles observed were 20-40 days in length, but irregular cyclicity was also apparent in both parous and nulliparous females, with short (<20 days) and extended cycles (>40 days) often exhibited over a 12-month period. Furthermore, within females, these extended cycles were associated with elevated faecal glucocorticoid metabolite concentration compared to other cycle types. In males, differences in faecal testosterone concentration were observed between males that had previously sired offspring, and those that had not, but this was unrelated to faecal glucocorticoid concentration. Potential correlates of the observed differences in reproductive and adrenal hormone concentration are being investigated, in an attempt to minimise reproductive skew and maximise the reproductive output of this population.

Improving the Welfare of Captive Asian Elephants in Kerala, India
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Improving the welfare of captive Asian elephants in India is emerging as an important conservation issue. Improving elephant welfare is one of the research priorities of scientists, policy makers and researchers. This study was done in Kerala state, the southern state of the country where elephants are used widely for festivals. Tuskers are used for processions and festivals in Temples and churches. Recent findings revealed that due to poor elephant management, 400 human lives have been lost during the last three and a half decades in India, of which more than 90 percent of the victims were mahouts. It is clear that poor elephant management practices cause elephant aggression toward mahouts.

This study was conducted in the southern state of India, Kerala which occupies only 1.13 percent of the geographic area of the country. The state has 600 captive Asian elephants. As part of the study, 100 captive Asian elephants were selected to determine the cause of elephant aggression and to formulate measures to improve captive elephant welfare. Variables like breeding, feeding, management, season, age, musth incidence, behavior, transportation and diseases were identified. Major interventions affecting the above variables were also identified and a SWOT analysis was done. Major indicators of poor elephant welfare include few chances for breeding, unscientific feeding and management, prolonged standing in festivals, unscientific musth management, poor musth forecasting system, heat stress, poor transportation and disease management.

Based on the above findings, a system was formulated to improve elephant management after taking into account the major variables identified including best feeding and management practices, musth forecasting system, scientific disease control system and best management practices during transport and festivals. The new scientific management system was administered on 100 captive Asian elephants during the festival season of 2012-2013. During festival season, when the elephants are compelled to stand more than 6 hours, measures were taken to frequently give watery vegetables like cucumber and watermelon. In order to reduce heat stress, the elephants were allowed to walk in shady places and wet gunny bags were placed underneath their feet. Shamianas were made where the elephants stand to protect them from scorching sunlight. A 12 hours rest period was made compulsory for all elephants before moving to the next festival. Mahouts were given training in scientific management practices. As part of transportation norms, elephants are allowed to walk only 20 hours per day during morning and evening hours. For travel of more than 20 km, trucks were made mandatory as per the state’s captive elephant management rule.
This study’s findings revealed that best captive elephant management practices could reduce 90 percent of the elephant aggression toward humans. In order to reduce stress, scientific feeding and management, disease control and management and attention to musth is required. There is a positive correlation between season and incidence of musth therefore a musth forecasting system will help to reduce elephant aggression toward humans and improve elephant welfare.

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Relationship Between Management, Adrenal Activity and Reproduction in a Captive Group of Female Asian Elephants (*Elephas maximus*)

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Asian elephants in captivity often exhibit low rates of reproduction and can be prone to reproductive problems including acyclicity. In order to maintain a healthy and sustainable population, a better understanding of social and environmental influences on reproductive cyclicity is warranted. Annual routine reproductive monitoring of faecal progesterone of five reproductive-age females revealed that oestrus cycles were highly synchronized among individuals. However, in one year, a 15-week period of acyclicity occurred in three of the five females, which coincided with a number of management changes, and the conception of two of the five females in the group. The aim of this study was therefore to retrospectively investigate whether changes in management factors were associated with increased adrenal activity and subsequent suppression of ovarian activity; or whether the conception of herd-mates might better explain cessation of cyclicity. Faecal samples (n=1032, ~200 samples per individual), collected every other day, were analysed for progesterone and glucocorticoid metabolites (as a reflection of adrenal activity) by enzyme immunoassay over 16-month consecutive period, to cover four potential oestrus cycles, including the observed 15-week period of acyclicity. Concurrently, management factors including training, matriarch presence and husbandry regimes were recorded daily. Data were analysed using General Linear Mixed Models for each individual to investigate whether faecal glucocorticoid metabolites were related to 1) Management [matriarch presence, training and foot care] or 2) Reproduction [individual reproductive status or the presence of other pregnant individuals]. Routine training and foot care were not associated with any change in adrenal activity for any individual; however, intensive foot care and intensive training were associated with an increase in adrenal activity in one female. Matriarch presence influenced adrenal activity in one sub-adult and three adult females, but not the juvenile female. In the three females that exhibited acyclicity there was no consistent relationship between faecal glucocorticoid metabolites and reproductive state; however, the start of the acyclic period coincided with the dominant female’s pregnancy and the conception of a second herd-mate. This study provided the physiological evidence (adrenal activity) to support prospective management changes including, no longer separating the matriarch from the group and refurbishing the indoor and outdoor enclosure with sand to improve foot health. This study highlighted how daily zoo management activity records and hormone monitoring can be used to support prospective management changes to improve the care and welfare in a group of Asian elephants.

Social and Reproductive Behaviour of Critically Endangered Northern White Rhinoceros (*Ceratotherium cottoni*) in a Zoological Garden

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The northern and southern white rhinoceroses have very low reproduction in captivity and social interactions between the animals, especially their increased agonistic behaviour, are believed to be one of the possible reasons. Free-ranging white rhinos have a network of social relationships with their conspecifics, which is, however, usually not available to them in captivity. Appropriate group composition and/or a change of social relationships in white rhino herds might therefore have a positive influence and increase the reproductive rate of captive rhinos. However, studies investigating the changes in group structure on the social and reproductive behaviour of captive rhinos are missing. The northern white rhino is currently on the brink of extinction with only seven animals known to survive. We studied the social and reproductive behaviour of a group of northern white rhinoceros (one male, five females) in zoological garden Dvůr Králové in 2005. The most often observed agonistic activities among the animals were threat, snarl and clash of horns. From the total number of agonistic activities in the herd, 73% was directed towards the male. In the middle of our study, one of the females (the oldest one and the only one wild-born) was separated out of the herd. Following her separation, agonistic behaviour among the rhinos significantly increased (p = 0.04). In addition, play behaviour, especially between the male and females also increased (p = 0.04). Play behaviour is, however, observed in adult male-female interactions in the wild only very rarely. We did not observe any changes in sociopositive behaviour (p = 0.79). Social dominance among the females, which might affect their reproduction, was not found. A presence of old and experienced female in the herd might have had a positive influence on the social interactions among other animals. Our results show that a composition of white rhino groups in captivity can have a significant influence on the social interactions among the rhinos. Better knowledge of appropriate composition of their groups in terms of age, sex and wild or zoo origin might therefore improve animals’ well-being and increase a chance for their reproduction.
Visual body condition scoring (BCS) is one of the most practical and effective tools for evaluating the general health status of an animal. This tool has been used to maximize reproductive success in production animals and identify health risks in domestic and exotic species (Gearhart et al., 1990; Laflamme, 2005; Lemma et al., 2006; Samarutel et al., 2006; Scarlett and Donoghue, 1998). Several visual BCS systems have been published for the Asian elephant (Elephas maximus) (Fernando et al., 2009; Ramanathan and Mallapur, 2008; Ramesh et al., 2011; Wemmer et al., 2006; Wijeyamohan et al., in preparation). Although each system uses a different scale, the relative descriptions are similar.

The Fort Worth Zoo (FWZ) has developed a 9 point BCS scale for the Asian elephant based on successful systems in other species which have been validated against direct measures of fat, correlated to health outcomes and used to manage captive populations. The FWZ system provides descriptions of standardized images (Figure 1) developed from an image database of hundreds of captive and wild Asian elephants in North America, Europe and Asia spanning the full spectrum of body condition (emaciated to extremely obese). These descriptors are being validated using direct measures of fat by transcutaneous ultrasounds collected from over 100 elephants in North America and Asia. Transcutaneous ultrasound is used in many domestic and exotic species to evaluate fatness and has been shown to correlate to visual body condition, although the strength of correlation may depend on ultrasound location and species-specific fat deposition (Domecq et al., 1995; Gentry et al., 2004; Wilkinson and McEwan, 1991). Therefore multiple ultrasound locations were tested for measuring fat in the Asian elephant in this study.

A subset of 12 elephants from 3 institutions in North America were sampled concurrently for visual body condition and transcutaneous ultrasound over rump and ribs (Figure 2). Four FWZ elephants were also sampled longitudinally for evaluation of body condition changes within individuals. Average elephant weight was 3200 kg (range 1491-4242), average BCS was 6.25 (range 4-9) and fat thickness ranged from <1 to more than 5 cm. Body condition score was shown to correlate significantly to ultrasound fat thickness.

Validation of visual BCS in Asian elephants using direct ultrasonic measures of fat supports the use of BCS as a tool for managing the condition of captive elephants and evaluating wild populations. Obesity in captive Asian elephants is suspected to be a significant problem associated with prevalent health concerns such as foot problems and reproductive failure. The combined body of work on Asian elephant body condition to date provides a strong, supported and practical management tool for improved health and success of captive elephant herds.

**Figure 1.** Standardized photoset for body condition scoring.

**Figure 2.** Standardized ultrasound locations over rump (from point of hip parallel to tailhead) and ribs (30% and 50% from point of hip to shoulder peak, down 10% of heartgirth).

**Literature Cited**


Body Condition Scoring Index for Female African Elephants Validated with Ultrasound Measurements of Subcutaneous Fat
Kari A. Morfeld & Janine L. Brown
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Several anecdotal reports suggest zoo elephants are obese, and that obesity-related health and reproductive problems are contributing to the non-sustainability of the African elephant (Loxodonta africana) population in U.S. zoos. However, a major constraint in screening for obesity is the lack of a practical method to accurately assess body fat in elephants. Body condition scoring (BCS) is the assessment of subcutaneous body fat stores based on visual or tactile evaluation of muscle tone and key skeletal elements, and provides an immediate appraisal of the degree of fatness of an individual. The objective of this study was to develop a visual body condition scoring (BCS) index for female African elephants and validate it using ultrasound measures of subcutaneous fat. To develop the index, standardized photographs were collected from zoo (n=50) and free-ranging (n=57) female African elephants to identify key body regions and anatomical sites that were used to visually assess body fat deposition patterns. The visual BCS method consisted of a list of these body regions and the physical criteria used for obtaining an overall score on a 5-point scale, with 1 being the thinnest and 5 being those with the most body fat. Significant correlations were observed between the visual scores and ultrasound measures of subcutaneous fat thickness at all anatomical sites, but were highest in the pelvic bone (r = 0.660, P < 0.01) and backbone (r = 0.664, P < 0.01) regions, indicating that BCS adequately reflects the amount of actual fat reserves. The new BCS index proved to be a reliable and repeatable method, with a high percentage agreement (73 % to 95%) and an overall "substantial" strength of agreement determined by the weighted k statistic (kw = 0.62 to 0.91) between and among three assessors that scored 40 elephants. In comparing photographs of wild vs. captive elephants, the median BCS in the free-ranging individuals (BCS=3, range 1-5) was lower (P < 0.001) than that of the zoo population (BCS=4). Results suggest that this new BCS index could be a valuable tool for examining the relationship between body condition and various factors affecting the health and reproduction of zoo and free-ranging elephants.

The Effect of Fresh Forages on the Fat Soluble Vitamin and Lipid Profiles of Greater One-horned Rhinoceroses (Rhinoceros unicornis)
Dr. Priya Bapodra1 BVetMed MSc, Dr. Ellen Dierenfeld MS PhD2, and Dr. Barbara Wolfe BSc DVM PhD Dipl. ACZM 1,3
1Columbus Zoo and Aquarium/The Wilds; 2Novus International, 3Ohio State University

The nutritional status of captive rhinoceroses has received many years of dedicated research, especially since nutritional aberrations in this taxonomic group are suspected to be linked to various disease syndromes (Clauss et al. 2002, Dierenfeld et al. 2005, Ghebremeskel et al. 1988). Previous research in the domestic horse has found that green forage is an important source of vitamin A derivatives and that captive diets provide lower amounts of vitamin E than native forage (Mäenpää et al. 1998, Dierenfeld et al. 1990, Dierenfeld et al. 1995, Ghebremeskel et al. 1991). Endogenous vitamin E levels have been shown to be affected by the level and type of dietary fat, antagonism of other fat soluble compounds, and additional oxidant stressors (Dierenfeld 1999). In addition, dietary concentration of vitamin E is the single most important variable affecting plasma a-tocopherol levels (Dierenfeld et al. 1997, Dierenfeld 1999).

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Management of a Breeding Herd of African Elephants (*Loxodonta africana*) on a Predominately Forage Diet

Ray L. Ball, DVM & Maura Davis

Tampa’s Lowry Park Zoo

The breeding herd of 1.3 African elephants at Tampa’s Lowry Park Zoo was transitioned from a traditional zoo diet consisting of large proportions of concentrates to one with predominately forage (hay and browse) with produce and concentrates used only as training items. Body weights were taken on a regular basis and two of the females have conceived while on the diet. Weight gains for the pregnant females have been relatively small, but still positive on this feeding plan. One calf has been delivered and this female has lactated normally. The female calf has shown good weight gain during the first 9 months of life. The second calf is expected mid-August 2013.

Blood samples on the adults were collected weekly for hormones analysis and serum banking. Retrospective analysis of essential fatty acids, minerals, and fat soluble vitamins was conducted on an opportunistic basis as sampling allowed. Comparisons are limited but can be made before and after the diet shift. Feeding forage only diet to African elephants appears to be beneficial as it has been in gorillas and white rhinos fed in similar fashion. Obvious benefits include weight management but more appropriate EFA may lead to better health of the digital cushion and hence less foot problems and increased feeding times can lead to less stereotypy. Consuming less fermentable feeds (grains) may also lead to less body heat generated during digestion and lessen the potential contribution to excess heat buildup. Less adipose will potentially lessen any effects of adipose derive hormonal affects (leptin) and less heat around gonads will decrease the chance of heat stress contributing to infertility.
Sex Differences in Captive Elephant Calf Social Interactions
Megan L. Houchin, Nicole M. Lyons and Robert H. I. Dale

It is not surprising that male and female elephant calves behave differently as they develop. However, this study took advantage of the opportunity to study a male and a female calf of similar ages (born 5 months apart) interacting in exhibit yards at the Indianapolis Zoo during a period when no other calves were present. In addition, five adult females, but no male elephants, lived at the zoo. This meant that the calves had no opportunity to observe male-female interactions involving older elephants. We observed both similarities and differences in the behaviors of the calves.

Asian Elephant Support: A Year in Review and the Importance of Collaboration
April Yoder & Linda Reifschneider

Asian Elephant Support (AES) is a registered U.S. non-profit foundation dedicated to the care and conservation of elephants in Asian range countries, and to the people whose lives are intertwined with this magnificent and endangered species. Over the past year, AES has participated in various conservation projects throughout Asia. The AES board of directors would like to share an update on these projects with the symposium participants. The presentation will include updates on the following projects:

• Collaborated with the Elephant Managers Association Conservation Committee to raise funds for the “Hoof Knives for Mahouts- India” program.
• Facilitated a grant from the USFWS to conduct a veterinary workshop entitled “Field course in Emerging Diseases” in India.
• Continued support for ElefantAsia by purchasing a portable scale for the Elephant Hospital in Laos.
• Hosted the 3rd Annual California Pizza Kitchen fundraiser.
• Provided emergency funding for orphaned baby elephants.
• Supported a project to document the relationship between elephants and mahouts.
• Continued support of veterinary workshops in Northern India (Assam).
• Supported EEHV testing in Sumatra, Indonesia.
• Facilitating a grant for a Veterinary Workshop in Myanmar- early 2014.

For more information and a list of the current Directors please visit our website at: www.asianelephantsupport.org.

POSTERS

Allomothering Among Captive African Elephants
Laura Beer, Heather R. Bates and Robert H. I. Dale
Butler University

According to P.C. Lee (1987), interactions between African elephant calves and other elephants are frequent, consisting of friendly, relaxed interactions or of adults providing assistance to calves. When a female elephant other than its mother provides comfort, care or protection a calf, it is called allomothering. Using video recordings of African elephants at the Indianapolis Zoo, we compared the interactions between several calves and Sophi (the “matriarch”/dominant female) and Tombi (a relatively subordinate adult in the group). Note that allomothering may occur either after an adult approaches a calf or after a calf approaches an adult. We examined the nature of the interactions between the calves and the adults.

Verbal Presentation Title: Sex Differences in Captive Elephant Calf Social Interactions
Megan L. Houchin, Nicole M. Lyons and Robert H. I. Dale
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The illegal trade in rhino horn is a leading contributing factor to the extinction of rhinoceros. Former evidentiary material can be used to further our knowledge base on how the horns are removed, processed, shipped, and modified for sale as whole horns or artifacts. Mechanisms of fakery can be detected using variable light source and low magnification to show how horse hooves and cattle horns are made into rhino horn imitations. Additionally, illegal fakes have been found made from carved elephant toenail. The implications of selling fake rhino horn include continued promulgation of the desire for rhino products and wasted time agencies/organizations spend in monitoring alleged contraband.

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All my life I have thought of myself as a problem solver and when the company I consult with was called into solve some issues with an elephant zoo enclosure I became fascinated with the many complex issues involved with keeping large animals safely and effectively in zoos, sanctuaries and game preserves. Learning more about the severe threat for the long-term survival of elephants and rhinos I truly wanted to help this cause. Loving a challenge, seeing the significant need, and after identifying enclosure issues with the keepers, I decided to develop some solutions. Because of my unique background with over 1000 gate systems that I have built and installed (including some very large ones), I have developed and built two scaled models that clearly demonstrate unique solutions for gates, fencing and ERD’s for large animals that also protects large animal handlers. The gate equipment is all concealled to be elephant-proof. The gates can move extremely fast in an emergency situation as they are built out of lightweight carbon fiber vs. heavy steel. I also have eliminated tracks in the ground where the animals pass through that presently requires daily maintenance to keep clear. The gate and the fence are built as modular units that get placed in a 2’ deep trench and bolted together, which allows for quick and easy installation. The fence, with its patented shallow footing is rated for an impact of 8 tons at 50 mph. The equipment can all be DC power, so that it can be installed in remote areas and run on solar power with a bank of 12V batteries that is mounted high for protection. All aspects of maintenance and installations are thus significantly easier, helping provide viable solutions for zoos, sanctuaries and game preserves.

Elephants for Africa: Conservation Through Research and Education
Kate Evans, Mphoeng Ofhilile, and Miguel Cases
Elephants for Africa

Rhinoceros Horns and Imitations in the Trade
Blount, V. M., B.C. Yates, E.O. Espinoza
National Fish and Wildlife Forensics Laboratory

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Elephants for Africa: Conservation Through Research and Education
Kate Evans, Mphoeng Ofhilile, and Miguel Cases
Elephants for Africa

Differentiation of *Mycobacterium* Species from Elephant Respiratory Samples in Nepal, Using Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP) Analysis
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Tuberculosis caused by Mycobacterium tuberculosis complex (MTBC) species poses a significant threat to the health and welfare of Asian elephants, and to the conservation of the species as a whole (Greenwald et al. 2009). Both *Mycobacterium tuberculosis* (M. *tb*) and *Mycobacterium bovis* (M. *bovis*) are endemic to Nepal, yet it is unknown which species are responsible for pulmonary mycobacteriosis in Nepal’s elephant population (NETCMAP 2011). Captive elephants interact closely with humans, wildlife and domestic species, increasing the risk for bidirectional transmission of *Mycobacterium* species and raising important public health, economic and conservation concerns (Murphree et al. 2011; Oh et al. 2002). It is widely accepted that, while early detection and management are essential for successful disease control in elephants, key inadequacies exist in current tuberculosis diagnostics (Angkawanish et al. 2010; Montali et al. 1998). Diagnosis relies on trunk wash (TW) culture and serology (Mikota and Maslow 2011). However, TW culture is impractical in many countries and lacks sensitivity (Mikota et al. 2006). Serology, while sensitive, does not differentiate the various *Mycobacterium* species, nor is it useful for monitoring elephants post-treatment (Kay et al 2010). The objective outcome of this research was to minimise the impact of tuberculosis on Asian elephant populations, through development of a novel molecular diagnostic technique, using Nepal’s captive elephant population as a model.

A gyrB-based PCR-RFLP assay for the detection of MTBC species was developed, building on preliminary work by Wilson and others (2008) at the Center of Molecular Dynamics Nepal (CMDN). The technique was found to be capable of detecting and differentiating *Mycobacterium tuberculosis* and *Mycobacterium bovis* DNA from elephant trunk wash and nasal drip samples. Preliminary genetic sequencing confirmed potential for future application for identification of drug resistance. Despite multiple limitations in relation to study protocol, field sampling and in-country laboratory and technical capabilities, it was demonstrated that the PCR-RFLP technique is practical, accessible and relevant to developing Asian elephant range countries such as Nepal, and provides a potentially valuable addition to the current array of diagnostic options. The findings and limitations of this research were collated to provide recommendations for future research and improved management strategies, to minimise the impact of tuberculosis on the wild and captive populations of this endangered species.
Preliminary Recordings of Wild Asian Elephant (Elephas maximus) Vocalizations in Preparation for Playback Experiments

Michael A. Pardo1*, Shermin de Silva, PhD2,3

Communication complexity is thought to increase with social complexity. Asian elephants live in a fission-fusion society and form multiple long-term social relationships, suggesting that their communication system may be particularly complex. They produce at least fourteen distinct call types1, but the meanings of these calls have never been experimentally determined. Growls (a type of low-frequency harmonic call) are individually distinct, but also highly variable within a given individual1. However, it is not known whether there are different structural or functional categories of growls. Combination calls are composed of a broadband segment immediately followed by a low-frequency harmonic segment1. Anecdotal reports suggest that elephants may respond more to combination calls than to low-frequency calls alone. The broadband segment may help call attention to the information contained in the low-frequency segment. Alternatively, the low-frequency segment may allow individual identity to be assigned to a broadband call. In December 2012 and January 2013, we recorded fifty-three calls from wild Asian elephants in Uda Walawe National Park, Sri Lanka, and categorized the vocalizations into basic call types according to de Silva1 by visual inspection of the spectrograms. Thirty-one calls were low-frequency signals with clear harmonics (growls and rumbles), eleven were broadband signals with no clear harmonics (barks, roars, and longroars), and five were combination calls (bark-rumbles). The remainder of the calls consisted of higher frequency sounds (trumpets and squeaks). We will record additional calls and pool our recordings with a pre-existing call library recorded by the Uda Walawe Elephant Research Project. We will then use cluster and discriminant function analysis to determine if growls can be subdivided into discreet structural categories. If structural categories exist, we will use playback experiments to test whether they correspond to functional categories. We will also use playback experiments to test multiple hypotheses for the function of combination calls.

ACKNOWLEDGMENTS

We are deeply grateful to the staff of the Uda Walawe Elephant Research Project, without whom this research would not be possible. We also thank the Sri Lankan Department of Wildlife Conservation for permission to work in Uda Walawe National Park. This project was funded by grants from the Cornell University Graduate School and the Athena Fund of the Cornell Lab of Ornithology.

References


Confirmation of the First Case of Endotheliotrophic Elephant Herpes Virus (EEHV) Infection in Nepal Using a Real-time Quantitative PCR Assay and DNA Sequence Analysis

S. Subedi, G. Hayward, S. Manandhar, C.A.J. Miller & D. Karmacharya
1 Center for Molecular Dynamics Nepal (CMDN), Kathmandu, Nepal
2 National Trust for Nature Conservation (NTNC), Sauraha, Nepal
3 Johns Hopkins School of Medicine, USA
4 Ecotone Wildlife Veterinary Services, Canberra, Australia

Elephant endotheliotropic herpes virus (EEHV) presents a growing threat to the health and conservation of both captive and wild endangered Asian elephant populations worldwide (Cracknell 2008). In the acute form the disease is characterized by a sudden onset of lethargy, oedema of the head, proboscis and limbs, oral ulcers, and diffuse internal haemorrhaging (Garner et al. 2009; Miller and Fowler 2012). In acute cases, death often occurs within one week following the onset of symptoms, and few cases have survived with intensive treatment (Hayward 2012). There is currently limited knowledge of the prevalence of EEHV in Nepal, a country with both captive and wild Asian elephant populations. However, since 2008, four elephant calves have succumbed to unconfirmed but presumed herpes virus infections (Gairhe 2012). Findings from a study of the Chitwan National Park (CNP) elephant breeding center in mid-2012 suggest the presence of latent EEHV infections in a number of adults within the breeding herd, although PCR and sequencing results were inconclusive (Kaufman et al., unpublished). In December 2012 a juvenile female elephant from the CNP breeding herd presented with ante-mortem and post-mortem changes characteristic of acute EEHV. Conjunctival swabs and heart, liver, kidney and blood samples were collected by the National Trust for Nature Conservation (NTNC) and analysed at the Center for Molecular Dynamics Nepal (CMDN). Samples were analysed using a validated real-time quantitative PCR assay (Hardman et al. 2010; Latimer et al. 2011), and all samples confirmed to be positive for EEHV. Using DNA sequencing and viral gene sub-typing analysis (Stanton et al. 2010; Stanton et al. 2012, in review) all samples were subsequently identified as subtype EEHV1A. These results mark the first PCR-confirmed case of EEHV infection in Nepal, and demonstrate the presence of EEHV in the CNP captive elephant population. Such findings support the hypothesis that latently infected individuals reside within the CNP breeding herd, and highlight the urgent need for adaptive management to minimise morbidity and mortality. Further research into EEHV prevalence, epidemiology and dynamics in Nepal are recommended in order to mitigate the impact of this disease on both captive and wild Asian elephant populations.

References


Hayward, G.S. Conservation: clarifying the risk from herpes virus to captive Asian elephants. Veterinary Record, 2012. 170: 8; 202-203.


Baby Steps
Kathy Suthard and Diane Hagey
Pittsburgh Zoo & PPG Aquarium

The Pittsburgh Zoo & PPG Aquarium celebrated the first black rhino birth in nearly half a century in September of 2012. The calf’s dam, Azi, was born at the Cleveland Metroparks Zoo, and the sire, Jomo, was born at the San Diego Wild Animal Park.

To insure that this calf would grow up self-confident and able to adjust to a variety of situations, the keepers drew from some traditional and not-so-traditional methods for working with rhinos. Initially the focus was on getting the baby familiarized with keepers working around her. Once she was comfortable with people being present, keepers began getting her accustomed to casual physical contact with humans. As she began to enjoy this contact, interactions were expanded to include selected techniques from Parelli™ Natural Horsemanship, Tellington TTTouch®, massage therapy, and operant conditioning. This was the beginning of a trusting relationship with this young animal and her keepers.

The successful interactions with the calf would not have been possible without the cooperation of Azi. The relationship building that began at the Cleveland Metroparks Zoo was instrumental in creating a solid foundation upon which Pittsburgh Zoo keepers could build. Azi’s calm acceptance of her keepers’ attentions was and still is a model for her calf.
PRESENTATIONS
SESSION 1
HUMAN-WILDLIFE CO-EXISTENCE
Securing Human–Elephant Coexistence

Simon Hedges

- Asian Elephant Coordinator | Ivory Trade Policy Analyst, Wildlife Conservation Society (WCS)
- Co-chair, IUCN/SSC Asian Elephant Specialist Group (AsESG)
- Technical Advisory Group (TAG)
  - CITES Monitoring the Illegal Killing of Elephants (MIKE) program
  - Elephant Trade Information System (ETIS)
Forest gardeners?
EWB has shown that the elephants of northern Botswana have the largest home ranges (24,828 km²).

These research findings contributed to delineating the Kavango-Zambezi Transfrontier Conservation Area (KAZA TFCA).
But... Asian Elephants now only occur in c. 10% of their historical range and many populations are now both small and isolated.
CITES
Appendix I (no legal commercial trade)

IUCN Red List
Asian Elephant (Asia-wide): Endangered (A2c; ver 3.1; Choudhury et al., 2008)

Sumatran Elephant subspecies (Sumatra): Critically Endangered (A2c; ver 3.1; Gopala et al., 2011)

…but how many Asian Elephants are there?
• Current ‘estimates’ suggest that there are 30,000–50,000 wild Asian Elephants in 13 countries.

• In reality these figures are little more than guesses (Duckworth & Hedges 1998; Blake & Hedges 2004; Hedges 2006; Hedges et al. 2009; Hedges 2012).

• Even the species’ range is not well known in some countries.

• Why? A general lack of scientifically rigorous monitoring.
IUCN Red List: Vulnerable

CITES: all populations in Appendix I except for Botswana, Namibia, South Africa, and Zimbabwe (Appendix II)
African Elephants are facing the most serious conservation crisis since 1989, when the trade ban was imposed by CITES (some argue that it is worse now than in the 1980s).

Why?

Largely because poaching is out of control

Populations and elephant ranges are collapsing

- Tens of thousands of elephants are now being killed every year
- Central African elephant population size “declined by ca. 62% between 2002–2011, and the taxon lost 30% of its geographical range. The population is now less than 10% of its potential size, occupying less than 25% of its potential range” (Maisels et al. 2013, PLoS ONE 8(3): e59469)

African elephants are now locally extinct in some areas and ecologically extinct in many more

Why?
CITES 2013, Bangkok:

- CITES 63rd Standing Committee Mtg, March 2nd
- CITES CoP16, March 3–14th
- CITES 64th Standing Committee Mtg, March 14th
STATUS OF ELEPHANT POPULATIONS, LEVELS OF ILLEGAL KILLING AND THE TRADE IN IVORY:
A REPORT TO THE CITES STANDING COMMITTEE

Introduction

In Decision 14.78 (Rev. CoP15), the Conference of the Parties directs the Secretariat to produce an updated analysis of MIKE data, and invites TRAFFIC to submit an updated analysis of ETIS data, the UNEP World Conservation Monitoring Centre (UNEP-WCMC) to provide an overview of the latest elephant trade data, and the IUCN/SSC African and Asian Elephant Specialist Groups to submit new and relevant information on the conservation status of elephants, and on pertinent conservation actions and management strategies, for consideration at the present meeting of the Standing Committee. This document has been prepared accordingly and is presented as an integrated piece of work to assess the status of elephants, levels of illegal killing and trade in ivory.

Decision 14.78 (Rev. CoP15) recognizes four global monitoring systems for elephants and trade in elephant specimens. The programme for Monitoring the Illegal Killing of Elephants (MIKE), managed by the CITES Secretariat, and the Elephant Trade Information System (ETIS), managed by TRAFFIC to track illegal trade in ivory and other elephant specimens, are mandated by the CITES Parties through Resolution Conf. 10.10 (Rev. CoP15). Thirdly, Article VIII, paragraph 7 (a), of the Convention requires CITES Parties to submit annual reports on the trade they conduct in specimens of CITES-listed species. These data, including legal trade in all elephant specimens, are compiled by UNEP-WCMC. Finally, IUCN, through the SSC African Elephant and Asian Elephant Specialist Groups, maintains the African and Asian Elephant Database, housing information on elephant population numbers and range.

Bringing together updated and critical information and data on elephants in an integrated manner, this report represents the second effort to provide information on the illegal trade in elephant ivory from a supply chain perspective to the CITES Standing Committee. Work continues to enhance and further develop linkages between ETIS, MIKE, and the IUCN/SSC African and Asian Elephant Specialist Groups. This integration is considered essential to support evidence-based decision-making for elephants in the CITES context.

Asian elephants (Elephas maximus): status, threats and conservation actions

This section draws on survey reports collected by the IUCN/SSC Asian Elephant Specialist Group (AsESG), the CITES MIKE programme, the Smithsonian Institution, the World Wide Fund For Nature (WWF), and the Wildlife Conservation Society (WCS) since the preparation of document SC62 Doc. 44.2 (Rev. 1). This section also considers recent research (both published and unpublished) to outline the status of and major threats to elephants in Asia, and the status of conservation strategies and actions. Curation of these data by the AsESG is underway and will allow the publication of an Asian Elephant Status Report, pending necessary funding.

IUCN Red List status

While the global status of Asian elephants in the IUCN Red List remains "Endangered" (A2c; version 3.1; Choudhury et al., 2008), the AsESG listed the Sumatran elephant (E. m. sumatranus) as "Critically Endangered" (A2c; ver. 3.1) in November 2011 (Gopala et al., 2011). The primary reason for the Critically Endangered listing was the scale and rate of habitat loss; taking ca. 25 years as a single generation (sensu IUCN, 2001) for Asian elephants, over 69 % of potential Sumatran elephant habitat has been lost within just one generation (see Figure A1 in document SC62 Inf. 1) and the driving forces that are causing the habitat loss are still continuing. Moreover, there is clear direct evidence from two Sumatran Provinces (Riau and Lampung) to show that entire elephant populations have disappeared as a result of the habitat loss over the past 25 years: nine populations have been lost since the mid-1980s in Lampung (Hedges et al., 2005) and a 2009 survey of nine forest blocks in Riau that had elephant herds in 2007 revealed that six herds had gone extinct (Desai, 2007). That this pattern will continue seems certain.
Devastating Decline of Forest Elephants in Central Africa

Fiona Maisels1,2,*, Samantha Strindberg1,*, Stephen Blake1,3, George Wittemyer1,3,4, John Hart1,3, Elizabeth A. Williamson1, Rostand Aba1,3, Gaspard Abital1, Ruffin D. Ambaye1, Fidël Ansindi1,3, Parfait C. Bakabana1,3, Thurston Cleveland Hicks1,4, Rosine E. Bayogo1, Martha Bechyni1,3,4, Rene L. Beyer1,3,4, Anietz N. Beanzogye1, Patrick Boudjou1,3, Nicolas Bout1,3, Marc Ella Akou1,3, Lambert Beno1,3, Bernard Bosso1, Elisabeth Greengrass1,2, Falk Grossmann1, Clement Bamba-Nkulu1,3, Omari Iambu1,3, Bibila Ingozibini1,3, Fortune Itunyengu1,3, Franck Kinoun1,3, Max Kokangaye1,3, Deo Kujirakwisha1,3, Stephanie Latour1,3,4,5, Inocent Liengola1,3, Quevenin Mackaya1,3, Jacob Madidi1,3, Bolia Madzoke1,3, Calixte Makumbou1,3, Guy-Almé Malanda1,3, Richard Malong2, Olivier Mban1,3, Valentim A. Mbenzyo1,3,4, Edgar Ambassa1,3, Albert Ekinde1,3, Yves Mihindou1,3, Bethan J. Morgan1,3, Prosper Motasba1,3, Gabin Moukala1,3, Anselme Mounguembu1,3, Brice S. Mowava1,3, Christian Nzazi2, Stuart Nixon1,3,4, Pele Nkumu1,3, Fabian Neolant1,3, Lillian Pintea1,3, Andrew Plumptre1,3, Hugo Rainey1,3, Bruno Bokota de Sembi1,3, Adeline Sercke1,3, Emma Stokes1,3, Andrea Turkalo1, Hilde Vanhee1,3, Ashley Vosper1,3,4, Ymke Warren1


Abstract

African forest elephants—taxonomically and functionally unique—are being poached at accelerating rates, but we lack range-wide information on the poaching. Analysis of the largest survey dataset ever assembled for forest elephants (80 field surveys, covering 73,000 km² in 90 percent-days of beltwaaks) revealed that population size declined by ca. 67% between 2002–2011, and the taxon lost 30% of its geographical range. The population is now less than 10% of its potential size, occupying less than 25% of its potential range. High human population density, hunting intensity, absence of law enforcement, poverty, and proximity to expanding infrastructure are the strongest predictors of decline. To save the remaining African forest elephants, illegal poaching for ivory and encroachment into core elephant habitat must be stopped. In addition, the international demand for ivory, which fuels illegal trade, must be dramatically reduced.


* These authors contributed equally to this work.

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Funding: No funding for the survey work was received from the academic or governmental or commercial. The African Wildlife Foundation, African Conservation Fund, Save the Elephants Foundation, ElephantVoices, Wildlife Conservation Society, Conservation International, Tree Aid, Transport Research Laboratory, Wildlife Conservation Society, Wildlife Trusts, and the University of Stirling. Fiona Maisels was supported by Wildlife Conservation Society while writing the manuscript. The Consejo Superior de Investigaciones Científicas (CSIC) provided logistical support during the field survey. The Department of Environment, Forests and Wildlife, Zambia funded this project. The African Wildlife Foundation and WCS provided logistical support to K. J. M. M. S.

Competing Interests: The authors have declared that no competing interests exist.

Received May 30, 2012; Accepted February 16, 2013; Published March 4, 2013

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ELEPHANTS IN THE DUST
THE AFRICAN ELEPHANT CRISIS
'White Gold'
A film produced by Simon Trevor of African Environmental Film Foundation (AEFF)
Presented at CITES CoP16 by Kenyan Wildlife Service (KWS), Kenya Elephant Forum, and Save the Elephants
If you buy ivory you kill people

Every year tens of thousands of elephants are being killed to feed the demand for ivory. Many people have lost their lives trying to protect the elephants.

Ivory is covered with the blood of elephants and people. If you buy ivory, you kill elephants and people.

The emotional intelligence of elephants can be compared to that of a four-year-old child. He understands what it means to kill a person. And you?

Stop the ivory trade!

elephantleague.org
So, what happened? Priority countries of concern (the so-called “Gang of Eight”) “put on probation”
The “Gang of Eight” were instructed to develop ivory trade action plans...

...with clear time-lines and milestones for specified actions

...have them approved officially by their governments by May 15th, 2013

...then implemented with compliance to be assessed up to the Standing Committee meeting in June 2014 (SC65)

...with the threat of sanctions for non-compliance
Annual reporting on stockpile inventories
Wildlife trade meeting endorses DNA testing of seized ivory

Protection for elephants, rhinos, sharks and trees extended in Bangkok.

Daniel Cressey
14 March 2013

Seizures of ivory will now be forensically analyzed to track the trade in illegal ivory.

If you go into a bar in Bangkok tonight, don’t be surprised if you find it full of celebrating conservationists.

HOW MANY ELEPHANTS ARE BEING KILLED?

Rampant poaching in the period between 1979 and 1999 reduced the elephant population across Africa from 1.3 million to fewer than 600,000 individuals, a loss of 7.4 per cent a year. Fast-forward to 2006, and the illegal ivory trade had once again increased to levels few people anticipated. Between August 2005 and August 2006, authorities seized more than 25 metric tons of ivory. Customs commonly assume that a 10 per cent seizure rate for “general goods” contraband is successful, and so the authors estimate that more than 250 metric tons of ivory were smuggled that year. Using the commonly accepted estimate of 6.6 kilograms of ivory per elephant, they conclude that 38,000 elephants — 8 per cent of the entire African elephant population — are being killed annually.

two to four nucleotides repeated from 10 to 100 times. Unlike functional genes, microsatellite DNA does not code for proteins. Thus, the number of repeats in the microsatellite can vary without affecting the health of the organism or its ability to reproduce, and changes in the repeat number tend to arise frequently and persist. Over time, then, microsatellites in one population come to differ among geographically separated populations.

With a DNA fingerprint of microsatellite repeats from a tusk in hand, we now compare this to a map of DNA fingerprints from elephants across Africa. A decade ago we set out to create a reference map across the whole of the continent that would plot the variation in microsatellite DNA. This project turned out to be a much bigger challenge than we anticipated.

Africa is a huge continent and the precision of our ivory assignments is directly tied to the comprehensiveness of our DNA map. To facilitate the collection of reference DNA, we extracted it from elephant populations in the least invasive way possible — through their feces. Each gram of elephant feces contains DNA from millions of sloughed-off intestinal mucosal cells. Collecting enough feces to create a reference map across Africa required the help of many scientists and wildlife wardens, and we are indebted to them for their efforts. But no matter how many elephant dung samples we are able to collect, we will never have enough to completely cover the entire continent.

To knit together swatches of patchy data, we use a statistical technique we devised called the smoothed continuous assignment technique (SCAT). Software employing the SCAT method extrapolates data gathered at discrete locations to compose a continuous geographic distribution of DNA fingerprints — the microsatellite DNA lengths at each of the 16 loci — across the entire elephant range. This method relies on the fact that populations close to one another tend to be genetically more similar than populations that are more distant. We validated the SCAT procedure by using it to determine the origin of dung samples taken from known locations.

Anatomy of a Shipments

We first applied our DNA assignment method to a case that exemplifies the magnitude and complexity of the modern ivory trade. In February 2002 authorities in Malawi, working with Zambian authorities and the Lusaka Agreement Task Force, raided a family ivory-carving factory that was ostensibly using ivory legally...
Iain Douglas-Hamilton on demand reduction, “we can now say with our hand on our heart that CITES supports campaigns to reduce the trade in ivory”.
What else was achieved? More attention paid to illegal trade in live elephants

- A growing problem especially in Myanmar
- At current harvest rate (c. 100 elephants/year) wild elephants could be extinct in the wild in Myanmar in 31 years

The captive (domestic / working) elephant population: has to be considered too

- Approx. 14,000 domestic/working elephants in captivity in range States
- Largest captive populations in Myanmar, Thailand, and India
- A resource (e.g. for research)
- A threat?
- Reintroduction to the wild?
High-level follow-up: Clinton Global Initiative (CGI) campaign

Partners’ Meeting, Central Park Zoo, July 15th, 2013 (WCS, WWF, TRAFFIC, Save the Elephants, CI, AWF, WildAid, IUCN, etc.)

CGI event at UNGA, Sept 2013

“Commitment” project over next 2+ years: range/transit States and NGOs working together to “Stop Killing, Stop Trafficking, Stop Demand”
Elephant Congress and Ministerial Meet (E:50:50) in India, Nov 14-19th, 2013

• Representatives of all 50 elephant range States will be present

IUCN African Elephant Summit, early 2014, Tanzania

London Summit on the illegal trade in ivory, rhino horn, great apes, and tigers, convened by UK Government, Prince Charles, & Prince William, in Q1 of 2014

NB: all before the July 2014 Standing Committee Mtg
What else is needed?
...well, the really important stuff...
We need stronger law enforcement at key elephant sites across Africa and Asia.
Informed by intelligence networks
Provide training, awareness-raising and support to judiciary, prosecutors, media

WCS Indonesia supported Wildlife Crimes Unit (WCU): a record of improving successful prosecution ratio, against a national background of 5%
Assigning African elephant DNA to geographic region of origin: Applications to the ivory trade

Samuel K. Wasser*, Andrew M. Shedlock**, Kenine Comstock***, Elaine A. Ostrander*, Benzeeth Mutayoba*, and Matthew Stephens*

*Department of Biology, Center for Conservation Biology, University of Washington, Box 351600, Seattle, WA 98195. **Biological Sciences, Fred Hutchinson Cancer Research Center, 1124 Fairview Avenue North, Box 13058, Seattle, WA 98101-2544. ***Department of Anatomy and Cell Biology, School of Medical Sciences, University of Auckland, Auckland, New Zealand. Edited by Henry C. Harpending, University of Utah, Salt Lake City, UT, and approved August 28, 2006 (received for review January 5, 2006).

Resurgence of illicit trade in African elephant ivory is placing the elephant at renewed risk. Regulation of this trade could be vastly improved by the ability to verify the geographic origin of tusks. We address this need by developing a combined genetic and statistical method to determine the origin of poached ivory. Our statistical approach exploits a smoothing method to estimate geographic-specific allele frequencies over the entire African elephants’ range for 16 microsatellite loci. Using 319 Kazuo and 84 suit samples from forest (Loxodonta africana cyclotis) and savannah (Loxodonta africana africana) elephants at 28 locations. These geographic-specific allele frequency estimates are used to infer the geographic origin of DNA samples, such as could be obtained from tusks of unknown origin. We demonstrate that our method alleviates several problems associated with standard assignment methods in this context, and the absolute accuracy of our method is high. Continental-wide, 93% of samples were located within 500 km, and 80% within 912 km of their actual place of origin. Accuracy varied by region (median accuracies: West Africa, 135 km; Central Savannah, 286 km; Central Forest, 414 km; South, 535 km; and East, 697 km). In some cases, allele frequencies vary considerably over small geographic regions, making much finer distinctions possible, and suggesting that resolution could be further improved by collection of samples from locations not represented in our study.

Materials and Methods

Samples. Mitochondrial and microsatellite DNA can be isolated and amplified from small amounts of African elephant ivory taken anywhere along a tusk (11). No attached tissue is required and old samples stored at ambient temperatures can be successfully extracted (13-15) samples and 262 forensic samples (Table 1), seven or more loci, and 75% of Africa's ivory in our sample (15). In Comstock et al. (14), one sample was found to be only 90% recovered for data and 1% State in compliance 3 and Plant Health

Extended to Asia
Social media campaigns to reduce demand for ivory in China and elsewhere
What are the (other) major challenges elephant conservationists face?

**MAJOR THREATS TO ELEPHANTS**
1. Illegal killing
2. Habitat loss / degradation
3. (The consequences of) human–elephant conflict
4. Population isolation / very small population size

**MAJOR CONSTRAINTS**
1. Lack of scientifically rigorous monitoring
2. Lack of capacity and political will in some countries
3. Insufficient funds
Habitat loss is the biggest threat to Asian Elephants and a significant and growing one for African Elephants.
Consequences of habitat loss include: Loss of entire populations.
Hukaung Valley Tiger Reserve

2004: 2,490 sq. miles
2010: 8,417 sq. miles
We also need to address isolation and very small population size issues: Still much to learn…

E.g., ongoing genetic research in Laos:

1. Still gene flow between Nam Kading, Nakai, and Seppon?

2. Habitat connectivity still extant or restorable?

Ahlering, Hedges, et al. 2011 (Conservation Genetics)
Human–elephant (HEC) conflict, driven in large part by habitat loss, is a major threat both to peoples’ livelihoods and to wild elephants.
HEC: finding the balance can be difficult...

This situation is no good! Too many people!

This situation is no better! Too many elephants!

Cartoon courtesy of TRAFFIC (ETIS)
Before...

After...
We need to know what drives HEC: e.g. are elephants major pests?

Cause of rice crop losses in 6 districts around Bukit Barisan Selatan NP, Sumatra (Hedges et al. 2005, Biol Conserv.)
We need to understand the full costs of HEC including opportunity costs:

- Farmers can only grow low value crops
- Guards lose opportunities to do other work
- Children too tired to go to school
Training staff how to assess crop damage as part of an evidence-based approach to HEC
Elephants hate chilli!
Testing HEC mitigation methods: burning ‘chilli dung’
‘Chilli-grease’ fences with cloth spacers
Coordinated crop guarding system:

Early-warning systems
Watchtowers
Crop-guards (2–3 guards per tower)
Spotlights
Walkie-talkies
Noise-makers
Successful reduction of HEC at a park-wide scale

From a WCS-funded pilot scheme in 1 village to...

...16 villages (that accounted for >90% of the HEC around Way Kambas NP) voluntarily adopting the methods and repelling an average of 74% of attempted raids

Hedges & Gunaryadi 2010 (Oryx)
Gunaryadi et al. In review (PLoS ONE)
Gunaryadi et al. In prep.
From research to policy-change… 
…Malaysia to move away from translocation for managing HEC
Monitoring is essential and we know how to do it...

333-pg peer-reviewed book, available free of charge, publ. 2012 (Funded by WCS, USFWS, & IEF)

Monitoring the Illegal Killing of Elephants

Dung Survey Standards for the MIKE Programme

March 2006 (rev 1)

Compiled and edited by S. Hedges and D. Lawson for the CITES MIKE Programme
The new IUCN African/Asian Elephant database

The Field

Web Exploration

Data Products

ELEPHANT DATABASE

Request process

analysis

web

email

Raw data

NGOs, students, researchers, etc.

AESR

AsESR in 2014
Thank you!
Endangered Species Act
Provides grants to strengthen local capacity to manage and protect wild populations of the target species.

- Rhinoceros and Tiger Conservation Act – 1994
- Great Ape Conservation Act – 2000
- Marine Turtle Conservation Act-2004
Most Recent Funds

2009: Critically Endangered Animal Conservation Fund

2010: Amphibians In Decline
Asian Elephant Conservation Fund
Update

From 2000-2013: ca. 397 grants awarded to ca. 115 grantees, all 13 range countries received grant awards from AsECF, to benefit wild Asian elephants and their habitats

FWS - $19,220,309 (approx)  Match - $25,596,674 (approx)

Photo credit: Ranjit Manakadan
Asian elephant range showing all Confirmed, Possible, and Recoverable range categories, and historical range

Range-wide Priority Setting Workshop for Asian Elephants
Elephas maximus. Final Report 98210-6-G232
Approximate population estimates
**Approximate population estimates**

<table>
<thead>
<tr>
<th>Country</th>
<th>Year 2008-11*</th>
<th>Previous count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sri Lanka</td>
<td>5879 DNP count in 2011</td>
<td>2500 in 1993</td>
</tr>
<tr>
<td>India</td>
<td>26-30,000</td>
<td>23,900-32,900 in 2005</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>253-332</td>
<td>196-227 in 2005</td>
</tr>
<tr>
<td>Bhutan</td>
<td>228-348</td>
<td>400-600 in 2005</td>
</tr>
<tr>
<td>Nepal</td>
<td>304-350</td>
<td>100-170 in 2005</td>
</tr>
<tr>
<td>Burma</td>
<td>1,231-1,492**</td>
<td>3-4,000 in 2005</td>
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<tr>
<td>Thailand</td>
<td>3-3,700</td>
<td>3-3,700 in 2005</td>
</tr>
<tr>
<td>Malaysia (Penin.)</td>
<td></td>
<td>766</td>
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<tr>
<td>Borneo (Malaysia)</td>
<td>1,500-2,000</td>
<td>1,100-1,600 in 2005</td>
</tr>
<tr>
<td>Indonesia (Sumatra)</td>
<td>2,400-2,800</td>
<td>1,180-1,557 in 2005</td>
</tr>
<tr>
<td>Indonesia (Kalimantan)</td>
<td>60-100</td>
<td></td>
</tr>
<tr>
<td>Cambodia</td>
<td>445**</td>
<td>250-600 in 2005</td>
</tr>
<tr>
<td>Laos</td>
<td>282 (700)</td>
<td>780-1,200 in 2005</td>
</tr>
<tr>
<td>China</td>
<td>117 (&lt;300)</td>
<td>200-250 in 2005</td>
</tr>
<tr>
<td>Vietnam</td>
<td>15 (&lt;100)</td>
<td>79-94 in 2005</td>
</tr>
<tr>
<td>TOTAL</td>
<td>48,472 approx</td>
<td>50,864 approx</td>
</tr>
</tbody>
</table>

* approx 75% of global population

** some areas unkn
International Conservation and Asian elephant conservation Threats and Challenges

- Demand
- Poaching
- Habitat degradation/loss
- Human-wildlife conflict
- Illegal/unsustainable trade
- Extractive industries
- Rapidly expanding human populations
- Lack of Law Enforcement
- Lack of capacity to manage and conserve wildlife
- Lack of appropriate policies and legislation
- Health/Diseases

Illegal logging in wildlife habitat
Home Guards and Volunteers strengthen security at Manas National Park, important for elephants, tigers, rhinos--India
Support for anti-poaching camps, patrol vehicles, and personal field equipment for forest guards
Capacity Building

Training Forest Officers

Training Teachers

Training Staff

Law Enforcement Training

Photo credit: Sally Walker

Photo credit: Simon Hedges

Photo credit: Sally Walker

Photo credit: Sally Walker
Capacity Building
Surveys, Monitoring, and Other Applied Research

Dung survey for population estimate

Photo credit: WCS
Human Elephant Coexistence training in Bhutan

Training teachers, forest officers, community members and leaders.
Now in India, Sumatra, Thailand
Conservation Response Units with the help of captive elephants and local communities in Sumatra:

Patrol forests and catch illegal loggers
Protect forests and wild elephants
Engage in community based conservation
Engage in eco-tourism
2084 wells closed!
Human-Elephant Conflict
Invasive species

Lantana camara
Encroachment into PAs by cattle

Consequences:
Diseases spilling over to wildlife
Habitat degradation
Human-wildlife conflict
Human-Elephant Conflict

Crop damage by elephant

Elephant killed in retaliation for crop depredation
Human-Elephant Conflict

Homes damaged

Fences around villages to protect people and property and allow elephants to roam the landscape
There are many conflict mitigation tools: Chili rope, Chili cloth, Chili and dung, Bee hives, Early Warning Systems...

For the “mitigation tools” to work, all of them require a concerted effort on the part of affected people.
Successful HEC project outside Way Kambas NP, Sumatra (grantee WCS):

- Organized guarding by community members
- Fish ponds at the perimeter
- Microfinance opportunities
- Great potential for replication elsewhere
Farmers coexisting with elephants - Bhutan
Mr. Tuy Sereivathana (Cambodia) with President Obama

Goldman Awardee 2010 and National Geographic Emerging Explorer Awardee 2011
Asian Elephant Program Funding 2005-2010

- Other: 3%
- Appliance surveys/monitoring: 24%
- Capacity building: 15%
- Conservation education/ community outreach: 11%
- Management plans: 3%
- Protection of at-risk populations: 6%
- Habitat/spp/PA management: 5%
- HEC: 18%
- Wildlife Health: 4%
- Law Enforcement: 10%
- Wildlife Trade: 2%
Asian Elephant Program Funding 2002-2011
Asian Rhino Fund
2013 Priorities for Asian Rhino Conservation

In FY 2013 the U.S. Fish and Wildlife Service's Rhinoceros and Tiger Conservation Fund provided $834,000 ($832,000 in matching funds) through 13 grants to benefit Asian rhino conservation.

Protection of rhinos and corridor between Bardia and Katarniaghat
Control invasive species in Chitwan and Manas
Asian Rhino Fund
2013 Priorities for Asian Rhino Conservation
Indonesia

Anti-poaching patrols: Javan rhinos at Ujung Kulon, and Sumatran rhinos at Way Kambas and Gunung Leuser NPs

Identify and protect important Sumatran rhino sites in Gunung Leuser NP

Investigate wildlife trafficking in vicinity of Bukit Barisan Selatan NP

Monitor Javan rhinos in Ujung Kulon NP with camera and video traps for information to establish a second population

Manage disease risks and improve livestock health to secure future of Javan rhinos
Train law enforcement personnel for more effective investigations and prosecutions of wildlife crimes in Thailand, Vietnam and China (ARREST)

Campaigns to reduce demand for wildlife products in Thailand, Vietnam and China (ARREST)

Identify gaps in legislation important to prosecution of poaching and illegal wildlife trafficking crimes for law enforcement, prosecutors and judiciary (ARREST)
2013

African Elephant

Appropriation $1.4m
+ Stamp $330,000
+ USAID TZ $200,000
2013

African Elephant

- Appropriation $1.4m
- Stamp $330,000
- USAID TZ $200,000

29 field projects in 11 African range states

2 projects on consumer side in Asia

1 core support to IUCN AfESG
2014: priority sites, part I

CENTRAL and WEST

• TRIDOM region
• Greater Sangha-Ndoki region
• Central African coast: Gamba complex-Loango-Moukalaba-Doudou-Mayumba-Conkouati
• Lope-Waka-Birougou
• Maiko-Okapi-Ituri forest
• W-Arly-Pendjari
2014: priority sites, part II

EAST and SOUTHERN

- Northern Botswana-Khaudum-Caprivi-Hwange
- Selous-Mikumi-Niassa
- Moyowosi-Kigosi/Ugalla/Katavi-Rukwa/Ruaha-Rungwa/
- Luangwa-Zambezi valley
- Laikipia-Samburu
- Greater Limpopo TFCA
- Tsavo-Amboseli-Chyulu
2013

Rhinoceros and Tiger
Appropriation $2.3m
Africa Rhino: 1/3=$772,000
+Stamp $110,000
+USAID TZ $100,000
2013

Rhinoceros and Tiger Appropriation $2.3m

Africa Rhino: 1/3=$772,000 + Stamp $110,000 + USAID TZ $100,000

14 field projects in 5 African range states

1 core support to IUCN AfRSG
Operation Crash

140 officers in 13 states
14 arrests, 6 convictions
so far
Operation Crash

140 officers in 13 states
14 arrests, 6 convictions so far

SEIZED: 37 rhino horns
$1m in gold, $1m cash, 2 vehicles
$683,000 for the African rhino program so far
The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

International treaty, 178 nations, protects species from becoming endangered or extinct because of international trade. Includes elephants and rhinos.

March 2013, member countries agreed on stronger actions to address escalating levels of poaching of elephants and illegal trade in elephant ivory

- Create Ivory Enforcement Task Force
- Increase monitoring and cooperative investigation of illegal ivory trade
- Adopt country-specific, time-bound actions for countries that are most often implicated as source, transit or consumer countries for illegal ivory trade
USFWS currently examining regulations, especially with regard to elephants and rhinos, to facilitate law enforcement actions and close loopholes that could be exploited for wildlife trafficking.
Steps forward, challenges...

- Reduce human impacts
- Demand Reduction
- Develop and Implement sensible land-use plans
- Ensure Law Enforcement
- Address environmental health
THANK YOU!

Acknowledgements

Grantees: for successful execution of projects, conservation of elephants, rhinos and their habitats, stellar photos

Range country governments: for conservation of natural resources and endangered species under challenging circumstances

Colleagues at USFWS: for proposal reviews, assistance in many ways!
Beehive fences as effective deterrents for crop-raiding elephants: field trials in northern Kenya

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Abstract

Increasing elephant populations in Kenya since 1989 have been widely praised as a conservation success story. However, where elephants and agricultural land overlap, incidents of human–elephant conflict are on the increase. Wildlife managers and farmers are now trying different farm-based deterrents to keep elephants out of crops. Here, we present data on the effectiveness of a novel beehive fence deployed in a Turkana community of 62 communally run farms in Kenya. Specifically, 1700 m of beehive fences semi-surrounded the outer boundaries of seventeen farms, and we compared elephant farm invasion events with these and to seventeen neighbouring farms whose boundaries were ‘protected’ only by thorn bush barriers. We present data from 45 farm invasions, or attempted invasions, recorded over 2 years. Thirteen groups of elephants approached the beehive fences and turned away. Of the 32 successful farm invasions, only one bull elephant broke through the beehive fences. These results demonstrate that beehive fences are more effective than thorn bush barriers at deterring elephants and may have a role in alleviating farmer–elephant conflict. Additionally, the harvesting of 106 kg of honey during the trial period suggests that beehive fences may also improve crop production and enhance rural livelihoods through honey sales.

Key words: African elephants, beehive fences, beekeeping, crop-raiding, farm-based deterrents, human–elephant conflict

Résumé

L’accroissement des populations d’éléphants au Kenya depuis 1989 a été largement salué comme une victoire de la conservation. Cependant, là où éléphants et terrains agricoles se rencontrent, les incidences de conflits hommes-éléphants sont de plus en plus nombreuses. Les gestionnaires de faune et les exploitants agricoles essaient aujourd’hui différents moyens dissuasifs pour garder les éléphants loin des cultures. Nous présentons ici les données sur l’efficacité d’une clôture originale intégrant des ruches déployée dans une communauté turkana comptant 62 exploitations gérées collectivement au Kenya. Très précisément, 1 700 mètres de clôtures avec ruches entouraient à moitié les limites extérieures de 17 exploitations, et nous avons comparé les incidences totales d’invasions par les éléphants par rapport à ces dernières et aussi à 17 exploitations voisines qui ne sont protégées que par des barrières de buissons épineux. Nous présentons des données portant sur 45 invasions, réelles ou tentées, enregistrées en deux ans. Treize groupes d’éléphants se sont approchés des barrières avec ruches et se sont éloignés. Sur les 32 invasions réussies, seul un mâle a traversé les barrières avec les ruches. Ces résultats montrent que ces clôtures sont plus efficaces que celles qui sont composées de buissons épineux pour dissuader les éléphants et qu’elles ont donc un rôle à jouer pour réduire les conflits entre exploitants agricoles et éléphants. De plus, la récolte de 106 kilos de miel pendant la période d’essai suggère que les clôtures avec ruches pourraient aussi augmenter la production des cultures et améliorer les moyens de subsistance ruraux grâce à la vente de miel.

Introduction

Conflict between farmers and elephants Loxodonta africana in Africa is becoming a notoriously complex problem to solve (Newmark et al., 1994; Hoare, 2000; Balfour et al., 2007). Both are competing for finite land and water resources (Sitati, 2003; Okello, 2005) in a
continent going through unprecedented human population growth. In Kenya, the government is keen to protect all wildlife as a national asset that also attracts much needed foreign exchange through tourism activities (Okello, Wishitemi & Lagat, 2005). However, there is intense media interest and political pressure to tackle the issue of human–elephant conflict (HEC) (Adams & Hulme, 2001; Omondi, Bitok & Kagiri, 2004; Balfour et al., 2007).

Electric fences have proved to be successful in barring elephants from some human-designated areas (Hoare, 2003; Kioko et al., 2008), but in Kenya, electrification projects have often failed because of poor maintenance, spiralling costs and/or a lack of community buy in (Thouless & Sakwa, 1995; Thouless, Georgiadis & Olwero, 2002; Okello & D’Amour, 2008). Much attention has been focused recently on the effectiveness of different farmer-based deterrents such as the use of buffer zones, fire crackers, dogs, watch towers or drums (Hoare, 1995; Osborn & Parker, 2003; Sitati & Walpole, 2006; Graham & Ochieng, 2008). Concentrated chilli extract burnt in dung bricks, sprayed or pasted onto string fences has also been tested as an elephant deterrent (Osborn, 2002; Sitati & Walpole, 2006). There has been considerable variation in the success and failure of these different mitigation methods across Kenya, and most HEC studies described here support each other by concluding that there is no one perfect deterrent, rather it is healthy to train and equip farmers with a ‘toolbox’ of various deterrents that, either combined or rotated, may have a greater effect than relying on any one method alone (Walpole et al., 2006; Hedges & Gunaryadi, 2009).

In this toolbox of deterrents, the African honey bee *Apis mellifera scutellata* could well be an important, and novel, component. First, it was shown that elephants avoid feeding on acacia trees with beehives (Vollrath & Douglas-Hamilton, 2002). This was followed by behavioural experiments demonstrating that not only do elephants run from bee sounds (King, Douglas-Hamilton & Vollrath, 2007) but also that elephants have an alarm ‘call’ that alerts family members to retreat from a possible bee threat (King et al., 2010). A pilot study using a beehive fence was found to be an effective elephant deterrent (King et al., 2009), but the scope of the study was small and the beehives remained empty during the trial. The concept of applying elephants’ natural bee avoidance behaviour to benefit rural farmers is an attractive one, not only could farmers benefit from reduced crop-raiding but such beehives could offer an additional income through the sale of honey and wax products.

Although a beehive does not ‘sleep’ at night, individual bees are less active as they can rest for several hours (Kaiser, 1988) and will spend time cleaning the hive and feeding the brood. Behaviour also seen during cold days (Hooper, 1997). Although such bee behaviour could be a limiting factor in the use of a beehive fence, as most elephant crop-raids occur at night, most elephant–man interfaces in Kenya tend not to be in cold/high-altitude zones. Additionally, there is a constant buzzing sound of bees fanning their wings from fully occupied *A.m. scutellata* hives, which may give elephants enough warning to stay away (King, Douglas-Hamilton & Vollrath, 2007). Furthermore, species of both Asian and African bees, *Apis dorsata* and *A.m. adansonii*, have been observed foraging successfully on moonlit nights (Fletcher, 1978; Dyer, 1985).

Here, we present evidence that beehive fences have a role to play as a novel farmer-managed elephant deterrent. Our data come from a 2-year participatory study (King, 2010) involving a Turkana community of 62 farms in northern Kenya.

**Materials and methods**

The farm-based trials were conducted in two small Turkana farming communities that have built up within the elephants’ range over the last 40 years (King, 2010). The communities are located 2 kms apart, within the greater Ngare Mara community, Meru North District in Northern Kenya (N 0.44529 : E 37.67353). Both communities have chosen to practice communal farming on either side of a rocky plateau on the lower flattened banks of two rivers that are less rocky and more suitable for agriculture.

The settlement neighbours three unfenced reserves, and communal farming strengthens the farmers’ ability to protect the crops from wild animals. Elephants in particular migrate between Shaba, Samburu and Buffalo Springs National Reserves and Meru National Park to the south (Douglas-Hamilton, Krink & Vollrath, 2005). Save the Elephants (STE) has recorded approximately 1200 elephants that use the three neighbouring northern game reserves, but the number of elephants utilizing the Ngare Mara area is unknown. Before our study, the area was identified by STE’s Monitoring of Illegal Killing of Elephants program (MIKE) as a hot spot for illegal killing of elephants (Douglas-Hamilton, Wittemyer & Ihwagi, 2010). Between

2002 and 2006, and prior to these beehive fence trials, nine illegally killed elephants were classified as ‘poached’ within an 8-km radius around this farming study site.

Multiple participatory community meetings were used to identify the layout of 62 farms and houses in the area. Each farm boundary and their positions were recorded and the GPS data uploaded into ARC GIS 9.2 (ESRI, Redlands, CA, U.S.A.) to create an accurate map. Using these maps, and participation from a focal group of ten men and nine women farmers, we identified the general routes most dominantly used by crop-raiding elephants. These historical routes revealed elephants coming to both rivers to drink and then walking up the opposite banks into the farms to crop-raid (Fig. 1). Although we could not verify this anecdotal evidence prior to the study’s commencement, this local knowledge helped us select all 34 farms that were ‘on the front line’ of these historical elephant raids. Beehive fences were constructed along 50% of the 34 most raided front line farms leaving the remaining 50% as control farms protected only by traditional thorn bush barriers. Each farm varied in size and therefore comparable lengths of farm boundaries were used to select bee (n = 17) and control (n = 17) farms rather than a purely random design (Fig. 1).

The beehive fences were constructed on the template of an earlier pilot design (King et al., 2009) but improved to include the more productive Kenyan top-bar hives (KTBH). Additional improvements came in the invention of a simpler, flat-thatched roofing system designed by a group of participating farmers during the construction phase (see Fig. 2). Three 80-cm-long beehives were constructed out of each 2.4 x 1.8 m, 9-mm industrial plywood sheet. The design of the KTBH hive (adapted from Jones, 1999) incorporates a queen excluder to keep the brood separate from the honey chamber, and this increases both the ease of harvesting and the value of the honey. Of these, 149

Fig 1 Map of the two focal farming communities Chumviyere and Etorro lying between the Ngare Mara and the Ngare Nite Rivers. All 62 farms in the two study farming communities were mapped with common routes used by crop-raiding elephants marked on as red arrows. Beehive fences were built along the boundaries of seventeen farms, and a further seventeen farms were classified as control farms where thorn bush barriers were left as the only defence between elephants and the field of crops.
beehives were constructed on site and deployed between June and August 2008 and the remaining 21 in April 2009 at a cost of US$22 per hive. Relying on data from a previous pilot study where elephants would not walk closer than four metres to a beehive (King et al., 2009), the beehive fences were built with one beehive to every 10 m. This resulted in 1700 m of beehive fences incorporating 170 beehives, around the boundaries of seventeen community farms, leaving any thorn barriers in place behind the beehive fences. A further 1700 m of farm boundaries were allocated as ‘control’ farms where just the thorn bush barriers were left in place along the seventeen farms. Two long stretches of farm land boundaries at the rear of the communal farm areas were not included as ‘controls’ because of this section was populated with houses and therefore identified by the community as an area too risky for elephants to traverse. All farms planted maize, often intercropped with beans, and they all relied entirely on natural rainfall.

Six farmers from both communities were trained to fill in simple data sheets detailing each farm and fence layout allowing the monitors to simply draw the movements of any elephants approaching or entering with details such as the time, date and number of elephants. These farmers worked on the farms daily and periodically walked around or slept near the farms at night during the crop-growing season. However, we could not measure guarding effort with any accuracy. Each farmer was given a personal beekeeping training session to help them manage their section of the beehive fence.

All farms were monitored over successive crop-growing seasons in an attempt to identify any variability there may be in seasonal differences in elephant behaviour. A farm invasion was defined as an elephant, or a group of...
Elephants, crossing a barrier (either bee or thorn) to enter a farm and later exiting either through this or through another barrier. If those same elephants chose to re-enter a second farm across a separate barrier, that second attempt was recorded as a second invasion. Elephants crossing into different farms within the communal area were not counted as separate farm invasions because of there being no internal barriers between the communal farming plots. Elephants that approached a barrier and turned away was a separate event, classified as ‘prevented from entering'.

Fig 3 Over 2 years, we observed that hive occupations closely followed rainfall patterns with peak occupations occurring during peak rainfall months. Honey harvests were poor during the first year and half of the project, but as occupations and rainfall increased, so did successful honey harvesting. Elephant events occurred mainly during harvest periods when rainfall resulted in successful crop growth. It was noticeable that elephants started to appear in the community at the same time that hive occupations were peaking.
farm’, even if those elephants were then to walk around and enter another farm at a different location on the same night.

We visited the study site once a week to help with fence maintenance and collect data on elephant raids, rainfall, maize growth, hive occupations and honey-harvesting events. Planting dates for each of the 34 front line farms were collected each season, and weekly maize growth rates were recorded in each farm by selecting three random maize stalks and taking an average of the three, which gave enough of an indicator of the condition of each farm over the passing weeks. This allowed us to test whether crop-raiding behaviour was biased towards riper fields. Rainfall was measured in millilitres by one farmer using a simple home-made rain gauge constructed from an inverted soda bottle. Although basic, this was an accurate enough intensity indicator of rainfall events. Data were analysed using Genstat v.11.2 (VSN International, Hemel Hempstead, U.K.) using nonparametric statistics.

Results

To identify seasonal variation in elephant-raiding behaviour, 34 farms were monitored over three crop seasons from June 2008 until June 2010. However, a harsh drought occurred in northern Kenya during most of the first year of the study period severely curtailing crop growth. Additionally, fatal tribal conflicts between our Turkana community and the neighbouring Borana tribe resulted in several farmers migrating during the trials and a consequent variation in planting success. Thus, the first crop season (October 2008–January 2009) saw only 18 days of light rainfall leading to failure of the harvest in 83% of the farms (Fig. 3). The second crop season (March–May 2009) had only 7 days of rain resulting in failure of the harvest in 100% of the farms. Finally, the third crop season (September 2009–February 2010) had 34 days of rain spread over 5 months resulting in a successful harvest of crops in 50% of the farms. Finally, the third crop season (September 2009–February 2010) had 34 days of rain spread over 5 months resulting in a successful harvest of crops in 50% of the farms, all in Chumviyere. Of the remaining farms, 38% failed to plant and 12% planted but the crops failed. Increases in beehive occupancy rates were closely associated with successful rainfall events, with poor occupancy occurring during the drought months of 2009, but high occupancy during the end of 2009 and early 2010 resulted in healthy honey harvests (Fig. 3).

Elephants visited the community between 29 April 2009 and 15 February 2010 when 45 elephant farm invasion attempts were recorded. Twenty-four farm events were recorded where elephants entered, or attempted to enter, and farms without crops (over a 259-day period) and 21 farm invasion attempts were recorded where crops were present (over an 82-day period) (Fig. 3).

We observed 32 events where elephant approached and were successful at invading the farms. Of these 32 invasions, 31 entries occurred through the thorn bush barriers, which was significantly different to the number of entries, n = 1, that occurred through the beehive fences (n = 31 (Thorn) vs n = 1 (Bee); \( \chi^2 = 28.125, \) df 1, \( P < 0.001; \) Table 1). In that case, an elephant, identified by the farmer as a bull, pushed through the wire connecting the hives thus avoiding the beehive huts. His family did not follow allowing the farmer to chase the bull back out. Both beehives on either side of the entry point were unoccupied at the time.

We observed thirteen attempted farm invasions where the elephants approached the beehive fences but did not push through. During eight of these thirteen events, the elephants (recorded by their footprints) walked alongside the length of the beehive fence structure, often approaching the wire within a metre or two and then backing away. However, in five events, the elephants walked along the

<table>
<thead>
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<th>Elephant behaviour</th>
<th>Farm condition</th>
<th>Thorn bush barrier</th>
<th>Beehive fence barrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevented from entering farm</td>
<td>No crops</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Entered farm</td>
<td>No crops</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Crops</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Exitied farm</td>
<td>No crops</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Crops</td>
<td>12</td>
<td>4</td>
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</tbody>
</table>

Analysis of 45 successful farm invasions showed that 31 invasions occurred through thorn barriers and only one through a beehive fence. A further thirteen attempts to enter a farm were deterred by the beehive fences, but in five of these cases, the elephants walked along the entire line of the beehive fence before breaking through a thorn bush barrier. Elephants exiting a farm after a crop-raid, or chased out by farmers, were more likely to exit through the thorn bush than the beehive fences. However, there were more observations of elephants exiting through the beehive fences than when entering a farm suggesting that elephants do not necessarily get trapped inside a protected farm by a beehive fence. Elephant behaviour and deterrent effects of the barriers were similar for elephants entering farms with or without crops.
entire length of the beehive fences until they came to the end of the line where they broke through the thorn barriers to invade a farm. At no point did we record elephants approaching the thorn bush barriers and turn away, every approach to the thorn bush barriers resulted in a successful entry to the farm. This result supports previous studies that thorn barriers are often ineffective barriers for elephants (Sitati, Walpole & Leader-Williams, 2005).

To further examine the effectiveness of the beehive fences, we analysed five successful crop-raids that occurred within the first 10 days of February 2010, the peak ripening time for maize in Chumviyere. In all five cases, the elephants broke into the farms at either end of Chumviyere’s 360-m beehive fence. We found no difference in mean maize height between the five neighbouring farms protected by the beehive fence (n = 5, mean height 229 cm ± SD 40.7) and the four control farms invaded at each end of the line of the beehive fence (n = 4, mean maize height 251.7 ± SD 25.9; Mann–Whitney U-test, U = 18.5, P = 0.647). This strongly suggests that invasions were not because of differences in crop attraction but because of differences in protection status.

Furthermore, in the 32 successful invasions, elephants also left a farm, both with crops and without, significantly more often through the thorn bush rather than through the beehive fences (n = 26 and n = 6, respectively; \( \chi^2 = 12.5, df 1, P < 0.001; \) Table 1). Indeed, in nine events, elephants already inside a farm walked along the inside of the beehive fences until reaching the thorn bush barriers where they pushed through to exit the farm. Nevertheless, in six events, elephants did run through a beehive fence when chased out by a farmer (Table 1). Of these six escapes, two occurred between beehuts where the wire had been removed by the farmer, three exits resulted in the wire detaching (as designed) and only once did the wire not detach effectively, and the occupied beehive was brought down. This beehive was successfully harvested by the farmer producing 8 kg of honey before it was repaired and rehung.

While the beehive fences protected the farms quite effectively, they also added to the productivity of a farm. Of the 150 beehives initially deployed around the community farms, 82 (55%) were occupied at least once between June 2008 and June 2010. A further 21 beehives deployed in early April 2009 had sixteen occupations (76%) up until the end of monitoring in June 2010 (Fig. 3).

During year one, we lost the honey from 38 occupied hives to suspected attacks by honey badgers *Mellivora capensis* over a matter of a few weeks. In response, we extended the protective iron sheets from 50 to 70 cm and since that design improvement, we only lost seven occupied hives to attacks by honey badgers. Additionally, we lost the honey from fourteen hives to suspected theft, but no beehives were stolen during the 2-year period.

Forty-four of the 98 occupied beehives were occupied more than once with some hives being occupied-abandoned-occupied as often as four times. Total occupation events within the 98 beehives were 169 revealing that previously occupied hives are more likely to attract a swarm. We observed that coating the beehives with a polyurethene-based varnish not only attracted scouting bees but also helped protect the plywood hives from weathering. Owing to high mortality rate of bees and comb from both the drought and from honey badger attacks, only 23 beehives were successfully harvested during the trial period. Nevertheless, the total weight of ‘elephant-friendly honey’ was 106 kg with an average of 4.6 kg per hive (range from 2 to 15 kg) at an estimated local value of US$290.

**Discussion**

Here, we present evidence that beehive fences can be a useful tool for deterring elephants from entering farm land. Analysis of 32 successful crop-raids demonstrated that elephants only once broke through the beehive fences to gain access to the crops within and that traditional thorn barriers offer no defence at all against such invasions. We recorded thirteen attempts to enter where the elephants turned away and either left the area after confronting the beehive fences or walked the length of the beehive fence to choose an easier entry point through the thorn bush. Additionally, elephants avoided the beehive fence boundaries when attempting to leave the farms after crop-raiding but if chased, an elephant could break through the wire to escape.

Recorded elephant avoidance behaviour occurred consistently along the beehive fences and in all cases, occupancy of the beehives could be regarded as low with just one or two hives occupied along the line. Despite low occupancy, the beehive fence as a novel, swaying, complex barrier appeared to successfully deter approaching elephants over a core study period of 10 months. As more approaches were made directly towards the thorn bush barriers (n = 31) than to the beehive fences (n = 13), it is possible that the elephants could either (i) see the beehive
fence swinging in the breeze as they approached from a distance or (ii) recognized the shape of the beehives and chose to re-orientate their approach to avoid the bees because of an expectation of a negative encounter.

More research is needed to understand how occupancy rates by live bees affect this decision-making process for elephants. As a result of the design of the beehive fence connecting the freely swinging beehives to each other with wire, the movement of one beehive actually causes up to three beehives on either side to swing. Is this physical, moving barrier alone enough of a deterrent? Does the three beehives on either side to swing. Is this physical, wire, the movement of one beehive actually causes up to connecting the freely swinging beehives to each other with elephants. As a result of the design of the beehive fence rates by live bees affect this decision-making process for because of an expectation of a negative encounter.

Additionally, there might be a saturation point in the number of beehives that can realistically be occupied in a certain area. One could hazard a prediction that the saturation point (if there is one) would be lower in the dryer semi-arid areas of Kenya where wild flowers and nectar abundance are more seasonal and droughts are more common. Saturation points might also be avoided by deploying some dummy beehives. Furthermore, what happens should every farmer in a community build a beehive fence? Would elephants simply walk around the farms and continue on their natural migration or would they become bolder and start to break through the fence where stretches of the hives remained unoccupied by bees?

The improved design of the beehive fence structure from previous trials (King et al., 2009) did prove effective, and maintenance was easier using the simplified flat-thatched roof. Kenyan top-bar hives improved the quality of the honey harvested from the hives as the honey was pure without brood) and attracted a good price at the local market. Farmers were quick to repair the damaged beehive fence from an exiting elephant, as they clearly understood the real and potential financial value that came from maintaining the fence. Although occupancy here was on the low side, the hope of any honey from the hives plus the protection from crop-raids appeared to be a real maintenance incentive for the farmers.

Despite the need for more specific research, the positive outcome of this study strongly supports the inclusion of beehive fences into the present toolbox of elephant deterrents to be trialed on a larger scale. Not only can such fences deter a significant proportion of crop-raids, but bees provide farmers with honey and other products for sale, which helps to diversify income. If combined with other partially effective deterrents, such as the use of guarding, dogs, drum beating or lights (Sitati, Walpole & Leader-Williams, 2005), or should chilli soaked grease be spread on the interlinking wires (Osborn, 2002), the combination of farmer-managed activities could create a successful elephant barrier that would be efficient, effective and be paying for itself over and above the rewards in arable products.

Acknowledgements

We thank the Office of the President of Kenya, Kenya Wildlife Service and Samburu and Isiolo County Councils for permission to conduct this research in Kenya. We are grateful for the cooperation and hospitality shown by all the families of Chumviyere and Etorro, in particular the carpenters Peter Ekerri and Charles Lobenyo. We thank Dr Anna Lawrence, Onesmas Kahiindi, Lucas Lepuiyapui and Wilson Lelukumani for research assistance. Funding was gratefully received from ESRC/NERC, Disney Worldwide Conservation Fund, Rufford Small Grants Foundation, Appropriate Development Consultancy Ltd and Save the Elephants. Additionally, we thank Save the Elephants for their research centre accommodation during the duration of the study.

References

Beehive fence field trials


(Manuscript accepted 26 May 2011)

doi: 10.1111/j.1365-2028.2011.01275.x

Using Bees as a Natural Elephant Deterrent

Dr Lucy E. King
Save the Elephants’ areas of research in Kenya
Tracking elephants to understand space needs and migration corridors
Mountain Bull’s hourly GPS tracking data
Damage to farms caused by crop-raiding elephants
Foraging in acacia trees can break open natural beehives.
Sensitive spots - the trunk, ears and eyes
Turkana Tribe
Hive Occupation Records

The table below should help you to design your own record sheets for monitoring activity around your beehive fence. Keep a record of every event including occupations, abandonment events, new occupations, harvesting events, visits by elephants or honey badgers etc. This system will help you identify sections of the beehive fence which are vulnerable to predation, drought, disturbance and which sections are providing you with the best honey harvests.

<table>
<thead>
<tr>
<th>Beehive</th>
<th>Hang date</th>
<th>Occupied date</th>
<th>Abandoned date &amp; reason</th>
<th>Harvesting date &amp;®</th>
<th>Event</th>
<th>Event</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.2.2010</td>
<td>18.4.2010</td>
<td>12.10.10 (left due to drought)</td>
<td>1.9.2010</td>
<td>occupied</td>
<td>3.2.2011 v. bad</td>
<td>bee bl 1</td>
</tr>
<tr>
<td></td>
<td>(example)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Top Tips to Improve Honey Production from your Beehive Fence

- **Keep a container of water topped up regularly near your beehives to encourage the bees to stay in their hives. Make sure you provide stones or sturdy branches in the water as bees will drown in deep water.**

- **Plant flowering plants around your beehive fence to provide nectar and pollen for the bees. Do not cut down any indigenous flowering plants as well fed bees will provide you with lots of honey.**

- **As a hive starts to fill with honey it becomes heavy and will start to pull down on the posts. If you don’t keep an eye on this, the hive will sag low enough for a honey badger to lean up onto the hive to feast on the honey. Replace any posts that are too weak to hold up a full hive.**

- **If you don’t have many beehives consider hanging ‘dummy’ beehives from some of the thatched posts – anything rectangle and heavy like an old piece of wood or a log. This will enable you to extend your beehive fence at little cost but whilst still retaining the swinging movement of the beehive fence system.**
Objective 4
Human-Elephant Conflict

Action 4.2.4
Trial deterrents
(e.g. disturbance methods, watch towers, fires, chili fences, *beehive fences*).
Niassa National Reserve, Mozambique – Niassa Lion Project
Queen Elizabeth National Park, Uganda – Malaika Honey
Publications

King (2012) Lambert Academic Publishing
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Vollrath & Douglas-Hamilton (‘02) Naturwissenschaften

www.elephantsandbees.com
With thanks to my project sponsors, partners and research team.
Elephants and rhino in Africa: Ban or market their products?

Twink Allen
The Paul Mellon Laboratory
Brunswick
Newmarket
Suffolk UK
The age-old trade
IVORY apocalypse

The African elephant is an instantly recognizable symbol of the natural wonders of our continent. The most mega of the earth’s terrestrial mega-fauna, these behemoths have stirred the imaginations, emotions – and adrenal glands – of humans for centuries. But our relationship with them is – and has been since before the time of the Romans – tainted by our obsession with their teeth. In pursuing the magnificent tusks that we carve and admire (and occasionally use), humans have slaughtered elephants in their millions.

After a brief respite following the carnage of the 1970s and ’80s, which halved the population, elephants are once again in the crosshairs. In an attempt to get to grips with the scale of the crisis, science editor Tim Jackson interviewed scientists, researchers, NGOs and policymakers across the conservation and trade spectrum, and filed this special report.

TEXT BY TIM JACKSON

by

Tim Jackson

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Poaching rampant in C & E Africa

1970

Annual legal ivory export from Africa 991 tons in 1976 680 tons in 1980

1980

400,000 elephant lost from E Africa

1990

CITES bans ivory trade

Kenya burns 12 tons of ivory

CITES sale of 50 tons to Japan

Trade slows due to CITES ban?

2000

One-off sales of ivory

2010

Drastic rise in poaching in C & E Africa

2013

US & Europe ban imports of raw and carved ivory

Prolonged financial recession in Japan - ivory use reduces

Meteoric rise in affluent middle class in China
2000

- Prolonged financial recession in Japan - ivory use reduces

2005

- Meteoric rise in affluent middle class in China

2010

- Majority of illegal ivory headed to China and Thailand
  - 60 tons ivory from Zambia seized in Singapore
  - China agrees to regulate ivory market
  - CITES allows SA, Namibia & Botswana to sell 60 tons ivory
  - CITES allows a record sale of 108 tons ivory. 9-year moratorium on further sales
  - CITES does not allow Tanzania or Zambia to sell ivory stocks in 2010
  - 2009 – 2011 Top years for seizures of illegal ivory
  - Tanzania withdraws application to sell 100 tons of stockpiled ivory

2013

- Ivory stolen from government stores in Botswana, Zambia & Mozambique

CITES allows SA, Namibia & Botswana to sell 60 tons ivory
Sources in Central Africa

20 yrs ago >100,000 Forest Elephant
Now, only 5 places left with <500

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in Central Africa

DRC

Gabon

>40,000 elephant still exist in the extensive rainforest.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in Central Africa

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)

<2,000 Savannah Elephant left in the north.
Sources in Central Africa

Population in the NE ~35,000 in the 1970s. Now <100.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in Central Africa

- DRC
- Gabon
- Cameroon
- CAR
- Chad

40,000 elephants 20 years ago. Now <200.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in East Africa

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)

Sudan

130,000 in 1986.
Down to <5000 today.
Lost 90% since 1980s.
Down to 1,200 in 2007.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in East Africa

Sudan
Ethiopia
Kenya

289 elephant poached in 2011; 384 in 2012.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in East Africa

- Sudan
- Ethiopia
- Kenya
- Uganda

Population fairly stable at 4,000. Poaching increased in last 4 years.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in East Africa

- Sudan
- Ethiopia
- Kenya
- Uganda
- Tanzania

Holds 80% of E. Africa’s elephant. Heavy poaching in last few years.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in Southern Africa

Around 20,000 elephant.
Little poaching so far.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in Southern Africa

- Namibia
- Botswana

± 130,000 elephant in 2012. Government resists poaching well.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in Southern Africa

Namibia

None poached since 2008 sale of ivory. Kruger now has 16,500.

Botswana

South Africa

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in Southern Africa

- Namibia
- Botswana
- South Africa
- Zimbabwe

90,000 counted in 2001. Little poaching but not well recorded.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Sources in Southern Africa

- Namibia
- Botswana
- South Africa
- Zimbabwe
- Mozambique

2,500 killed in north since 2009. Little enforcement action/capacity.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Out of Africa

Many illegal trafficking routes criss-cross Africa. E.g. Togo, Sierra Leone and Ivory Coast have few elephants yet all are active in the ivory trade.

Ivory now air-freighted – as much as 800 kg a shipment. All large scale seizures appear to be heading to China.

China has upped its law enforcement on ivory imports – and so the criminal syndicates have adapted (Tom Milliken).

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
The Asian dilemma

**Thailand** has one of the largest unregulated ivory markets in the world. In China, ivory is consumed by the Chinese which means it remains in China (Tom Milliken).

**Japan** is a story of demand reduction. In the 1980s Japan consumed >300 tons/year. Now it uses only 5 – 10 tons annually from legal stocks. Japan’s strict government regulations act to deter ivory carvers and vendors (Tom Milliken).

**China** displays ivory as an ostentatious form of wealth. In theory China has a regulated Ivory market but in practice the system is widely abused. Unlicensed ivory facilities in China outnumber legal ones by 6 to 1 (Peter Knight; IFAW).

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Guarding the assets

Tanzania holds >100 tons of ivory; Zimbabwe holds around 50 tons. All from natural deaths or seized from poachers.

In 2012 stockpiles raided and ivory stolen in Tanzania, Zambia and Mozambique. Also in 2012, Kenya and Gabon burnt government-held stocks of ivory.

Throughout Africa protecting elephants is being given higher priority. But corruption is a major issue across the continent.

From: Tim Jackson (2013) Ivory apocalypse. Africa Geographic (April)
Human population influence

At the turn of the 19th century Africa’s population was 100 million; today it is >450 million.

“People were once an island in a sea of wildlife. Now wildlife survives in Parks that are islands in an ocean of people.”

Raymond Bonner, 1996 ‘At the Hand of Man’

“Human increase and elephant decrease are two poles of the same phenomenon. The decline of elephants, rhinos and large mammals generally in East Africa has everything to do with human increase and displacement by people and relatively little to do with the value of their products.”

Ian Parker, 2010 ‘What I Tell You Three Times Is True’
The South Africa – Viet Nam Rhino Horn Trade Nexus

A TRAFFIC Report by Tom Milliken and Jo Shaw

South Africa – The main source
South Africa – The main source

SA now conserves 18,800 White Rhino (~25% are privately owned) and 2,350 Black Rhino (most privately owned).

Since the 1960s, a marked shift towards wildlife-based land use amongst private landowners. Today, game ranches areas are 3 times bigger than the collective national game areas.

South Africa – The main source

Trophy hunting has been a positive force for rhino conservation. It contributes to bio-management, range extension, revenue generation and incentive for wildlife conservation.

South Africa – Laws and policy

Vietnamese nationals have exploited SA’s legislation in order to obtain hunting trophies for the Asian rhino horn trade.

Rhino hunts are nowadays restricted to one hunt/hunter/12 months and government personnel must witness each hunt.
Illegal trade in rhino horn


Law enforcement

High calibre rifles, dart guns and helicopters being used to poach rhino, reflecting the involvement of game industry insiders, e.g. game ranch owners, professional hunters, game capture operators, pilots and wildlife vets.

Corrupt officials provide poaching gangs with intelligence. Private owners reluctant to report information on rhino numbers and stockpiles of horn.
Law enforcement


43 documented arrests of Asians for rhino crimes in SA; 56% Vietnamese and 28% Chinese.

African nationals caught poaching rhino get 10 years jail. Couriers transporting horn out of Africa get 6 – 12 years.

The end result

A potent mix of unscrupulous wildlife professionals, some corrupt government officials and hardened Asian criminal syndicates has converged to create “the perfect storm” for wrecking havoc on South Africa’s rhino populations.

Tom Milliken and Jo Shaw
Rhino horn: Uses in Asia

Much of the ‘rhino horn’ on the market in Vietnam comes from Water Buffalo. Overall, ± 90% of rhino horn products, especially jewellery items, are fake (Ammann 2012).

Local demand for rhino horn far outstrips supply so that well known traders have months-long waiting lists for the genuine substance (Barton 2012).

Like China, the younger generation in Vietnam is fuelling a booming market for luxury products. The rhino horn trade can be viewed as another aspect of the luxury products trade.

Rhino horn: Users in Viet Nam

**Habitual/social users** - Affluent, middle-aged urbanites imbibe rhino horn as a detoxifying beverage after drinking and bingeing.

**Elite gift givers** - To curry favour and gain influence. Many horns purchased and offered as high value and status-conferring gifts.

**Protective mothers** - As a medicine to treat pyrexia in children. They firmly believe it to be better than any Western medicine.

**Seriously/terminally ill** - Looking for a panacea. Dealers and touts actively seek out these people.
On the other hand ....

South Africa is still breeding more White Rhino annually than the number being poached.

E.g. John Hume has accumulated >850 White Rhino in the past 10 years.
On the other hand .. deer velvet industry

A traditional Chinese medicine – used as a growth tonic for children and to treat arthritis and other geriatric conditions. Dried and sliced for sale - boiled and mixed with herbs and consumed as tea.

New Zealand is the world’s largest producer of velvet – 450 tons annually to China.
On the other hand

Too many elephants in some locations.

E.g. Botswana > 130,000
Zimbabwe > 90,000
Kruger Park > 16,000

Hwange Park, Zimbabwe. November 2012

Relocation?
Imunocontraception?
Culling and product use?
Possible solutions

- Convince Asians to stop using rhino horn and ivory.
- Continue to hammer poachers, couriers and dealers.
- Allow controlled legal sale of stockpiled, farmed and hunted rhino horn and ivory with equitable distribution of profits to producers and wildlife conservators.
Utopian dream

A well organised and controlled, transparent pan-African marketing system to sell ivory and rhino horn to Asian user countries with fair distribution of the income to all those involved in creating it, especially indigenous Africans.

Barriers

Greed, jealousy and corruption at individual, institution and state levels.

CITES – the ultimate ‘elephant in the room’.
CITES – the unworkable treaty?

In 1979 Ian Parker noted ... “As more countries accede to CITES ... it will become evermore unwieldy. ..... While the motivation behind it is straightforward, ... it is too ponderous and impractical to succeed as it is.”

In 2010 he further noted that .... “...the 2-yearly International Meeting of the Parties .... is an unnecessary and wasteful jamboree.”
Why should southern African countries, like Botswana, Zimbabwe and South Africa, put up with being told what they may or may not do with their wildlife by countries like Norway, Nicaragua and New Zealand?

CITES – the unworkable treaty?
INTERNATIONAL ELEPHANT & RHINO CONSERVATION & RESEARCH SYMPOSIUM


Jim Nyamu- 26th August 2013
Mission:
To experience a landscape where elephants and communities are roaming widely and peacefully and in harmony with local economy and ecological environment.

Strategic vision:
To sustainably conserve and manage the graceful elephants and their home ranges in partnership with communities and other stakeholders for the present and future generations.

Our core values:
At the Elephant centre; we disseminate valuable knowledge, facilitate research, conservation and management of the elephants and their natural environment responsively and sustainably. We do this with the duty of care, integrity and inclusiveness.

Areas of operation
Samburu, Laikipia, Meru, Tsavo Ecosystems, Maasai Mara and Loita
What is ENC doing?

• Established **Community Based Elephant Monitoring Program (CBEMP)** in Loita with plan to replicate in other areas
• Working with youth from all the range lands
• Planning for National Youth Elephant conservation Forum
• **IVORY BELONGS TO ELEPHANTS CAMPAIGN**

www.elephantneigborscenter.org
AFRICA

- Habitat loss and fragmentation
- Human Elephant Conflict
- Poaching for meat and ivory
- Negative localized impacts on elephants and their habitats
- Debate over legalization of the international trade in ivory which divides countries holding
• Elephant numbers vary greatly over the 37 range states
• African elephants have a sub—Saharan distribution, with forest elephants primarily inhabiting western and central regions of Africa where relatively large blocks of dense forest remain
• Savanna elephants inhabit the eastern and southern regions with the highest densities found in Botswana, Tanzania, Zimbabwe, Kenya, Zambia and South Africa.
<table>
<thead>
<tr>
<th>Regions</th>
<th>Numbers</th>
<th>Range area(\text{km}^2)</th>
<th>% of continental Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Africa</td>
<td>10,383</td>
<td>975,079</td>
<td>29</td>
</tr>
<tr>
<td>Eastern Africa</td>
<td>137,485</td>
<td>880,063</td>
<td>26</td>
</tr>
<tr>
<td>Southern Africa</td>
<td>297,718</td>
<td>1,305,140</td>
<td>39</td>
</tr>
<tr>
<td>West Africa</td>
<td>7,457</td>
<td>175,545</td>
<td>5</td>
</tr>
</tbody>
</table>

Total: 453,043 \(\text{elephants}\) out of 3,335,827 \(\text{elephants}\) in total.
• Human-elephant conflict remains to be among the most prominent issues affecting elephant conservation in Kenya.
• The demand for ivory has seen a very large number of elephants massacred for their tusks in Kenya.
• Elephant tusks are used to make jewellery, carvings, sculptures, piano keys, enamel plates and billiard balls.
• Believe to cure health conditions associated with health and others use it as an aphrodisiac
• In 2003 Kenya embarked on a process to amend its current wildlife law, but the process has been hampered by controversy by stakeholders.
Where are the elephants in Kenya

- Mountain Areas Mt Kenya, Laikipia  -41%
- Tsavo Ecosystem … East and west  -35.0%
- Central Rift- Mara , Mau Ecosystem -11.6%
- Southern Area Amboseli Ecosystem  -4.8%
- Coastal areas. Shimba Hills, Arabuko -3.4%
- Western Areas, Mt Elgon, Kamnalok  -2.1%
- Northern Areas Garrisa ,Meru ecosystem -1.9%

80 % of Kenyan elephant are out side protected areas

www.elephantneigborscenter.org
National Elephant Strategy 2012-2021

Strategic Objectives

- Population expansion and Habitat maintenance
- Research and Monitoring for Management
- Human-Elephant Conflict
- Incentives
- Capacity Building
- Coordination and Support

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IVORY BELONGS TO ELEPHANTS
OBJECTIVES OF THE WALK

• To create more Awareness on the Importance of our Elephants Alive
• emphasizing on the need not to buy ivory products
• Resource mobilization for CBCP
• 123 High schools
• 90 Primary schools
• 16 churches
• 3 Mosques

• 56 CFA’s
FINDINGS

• 89.9% (= 2001) have no information on the effect of poaching

• 73.5% (= 2001) Do not like wildlife and in particular Elephants

• Out of (= 2001) 67% were youths who are ready to support KWS if they see any benefits

• 81.7% (= 2001) Feels that KWS should be responsible for the killings of the elephants
CONCLUSION

• There is a general sense of ignorance among the public with matters pertaining to the need to have elephants conserved.

• Communities are willing to tolerate elephant on their land provided that there are sustainable Human-elephant conflict mitigation measures.

• “We are willing to support ELEPHANT conservation if we can also share the benefits they bring to our country” a resident

• Over 70% of the community are against ELEPHANT poaching and call upon ALL stakeholders, International, National and Local intervention.

www.elephantneighborscenter.org
WAY FORWARD

Strategic Focus

• Evidence based advocacy- walking the Talk
• Skill development, capacity building and education
• Community participation-communities based conservation programs and projects
• Research and conservation
• Resource mobilization.
• Market based module- eco-lodges and tourism
USA Ivory belongs to elephant campaign walk

- Start on 3rd Sept from Boston-Washington DC 900km (International Elephant March)
- Kampala (Uganda – Dar eer Salama Tanzania – Amboseli Walk (JAN 214 (2800Km))
- China-Japan walk- April 2014
Thank you

www.elephantneigborscenter.org
PRESENTATIONS
SESSION III
IN SITU POPULATION DYNAMICS AND CONSERVATION

S.P. Downie* and A. Mavrandonis, B.Sc. Pharm

**ABSTRACT**

In 1989, South Africa had less than 20 *Diceros bicornis bicornis*. This project alone has helped quadruple that number. *The Biodiversity Management Plan for the Black Rhinoceros in South Africa 2011 – 2020*¹, (BMP; Government Gazette, 25th January 2013), calls for 260 by 2020, and a longer term goal of 500.

This project, registered with South African National Parks,² supports a number of strategic objectives of rhino monitoring and management by studying separate sub-populations in four smaller parks within their historical range. The accumulated data assesses and measures performance towards the BMP goals.

Although data is available for over 21 years, and the first founder population was introduced to one study area in 1999, regular ground monitoring commenced in 2002, and numbers have increased from 5 to 72 rhinos. All rhinos are individually identifiable. The objectives were to monitor developmental stages, study all performance indicators, assess dispersal into new habitats, determine success/failure of translocation strategies, study behaviour and develop genealogy.

The experience with Shibula, returned to southern Africa from Lisbon Zoo in 1991, has been an inspiration, was invaluable, and is comprehensively documented.

Monitoring involved locating, identifying and observing individuals using a combination of methods and techniques: telemetry, tracking spoor, searching from vantage points and once located, a spotting scope used to identify individuals by means of unique ear-notches.³

Accomplishments included 133 field trips, over 1,000 days in the veldt and over 1,300 hours of rhino observations. Over 80,000 photographs were taken and 104 hours of video were filmed. On average, we identified at least 75% of the rhinos per field trip, attempted to locate 100% each year, and during 80% of sightings ensured rhinos were undisturbed and therefore observed natural behaviour and social interaction.

Average annual growth rate was 15% over 14 years in one area with the most rhinos. The study highlights the problems of developing small populations, and the impact of management interventions. Population constraints were forecast and relocations recommended. As poaching reached record levels in 2010, emphasis moved to focus on safety and security, which led to the project initiating specific plans, supplying equipment, manpower and anti-poaching training.

This study highlights the success of undisturbed populations, careful unobtrusive monitoring and the importance of security. These four sub-populations will contribute significantly to the overall growth and survival of the sub-species, and exceed by far the growth targets set by the BMP. The project methods have proved to be a valuable formula for the future.

This monitoring and security project was funded by the David Shepherd Wildlife Foundation (DSWF) and the authors personally.
INSPIRATION – SHIBULA

“Our dedication to black rhinos was inspired by Shibula in 1991 – a very special black rhino. Her return to Africa from Lisbon Zoo in Portugal, adapting to a new wild habitat and giving birth to eight calves is an all too rare good news conservation story.”

Shibula changed our lives. She was gentle and inquisitive and allowed us to get close to her and her first two calves. We followed her progress from the concrete enclosure in Lisbon Zoo, to her introduction to a wild population to the birth of her first calf, Dundi, to their separation and relocation to different parks, to the death of two of her male calves, to the birth of her eighth calf. Shibula was the motivation for us to give back and dedicate our lives to do whatever we could to help her sub-species survive. She is now completely wild.

INTRODUCTION

The Black Rhino Monitoring Project in South African National Parks, 2002 - 2012

Our objective in documenting data over ten years has always been to compile and analyse accurate information on these sub-populations of the south western black rhino Diceros bicornis bicornis (D.b.b.), (hereafter referred to as rhino).

Shibula focused our conservation efforts on black rhinos in 1991. With very little knowledge, our learning curve was very steep, finally eliciting the following note from Dr. Michael Knight, Chairman of the AfRSG: “In this case the DSWF monitoring project managed by Sue Downie and Lucky Mavrandonis is of utmost importance to the individual parks, SANParks in general, and myself as Chair of the SADC Rhino Management Group and IUCN SSC African Rhino Specialist Group. Lucky and Sue’s unwavering dedication and attention to detail surpasses any other such rhino monitoring project in SANParks, and dare I say for any other state protected area in the country.”

The Black Rhino Monitoring project (BRMP) could be a guideline for what is needed to protect this species - private focused involvement, private funding, independent analysis, many man-hours physically spent observing rhinos, area-specific rhino security plans, plus the passion to commit to a long-term project. We have been privileged to have had the opportunity of “walking with black rhinos” for many years.

Comments from Dr. Anthony Hall-Martin confirm this essential role of the private sector: “It is now more than twenty years since Sue and Lucky began to work on the conservation of the black rhinoceros in South Africa ..... These efforts have made large new areas of habitat available for the southwest arid zone subspecies of the black rhinoceros, Diceros bicornis bicornis. On their watch a handful of these animals have grown into thriving populations ..... The monitoring of reintroduced black rhinoceros populations by Lucky and Sue has produced an impressive body of biological information of great value to science and to practical conservation.”

We believe that this study gives practical details for the successful implementation of projects to ensure D.b.b. numbers reach the Biodiversity Management Plan for the Black Rhinoceros in South Africa1 (BMP) targets set in 2013.

OBJECTIVES

The objectives of this project, a registered research project with SANParks2 were to:
1) assess the population performance of the arid adapted ecotype of the black rhinoceros sub-species *D.b.b.* under management of SANParks in four parks,
2) facilitate the development of appropriate management strategies,
3) assess dispersal into new habitats post-release,
4) fund and assist in the implementation of individual security plans, and
5) since the publication of the BMP in 2013, evaluate how these sub-populations can assist South Africa in achieving the short and long term goals of the Plan for *D.b.b*.

**STUDY AREAS**

*For security reasons, the four project areas are not identified by name, but by the first four letters of the Greek alphabet (Figure 1).*

1. **Alpha** is an arid area of about 44’000 ha with *noorsveld* type vegetation in the Karoo Biome. Euphorbia coerulescens or *noors*, a nutritious plant with a high fat content, is one of the major plant species browsed by the black rhino. After 2006, the area available to the rhino was about 35,000 ha and was home to 34 *D.b.b.* in May 2012, and probably 38 by December 2012.

2. **Beta** is situated on the northern slopes of a mountain range, located in the transitional area between four biomes: grassland, Nama Karoo, thicket and savanna, vegetation types which are poorly conserved in South Africa. A study by Brown & Bezuidenhout found twelve major vegetation units in the expanded national park. The areas mainly utilised by the rhino are the *Pentzia globosa – Eragrostis obtusa* shrubland and the *A. Karoo – Rhus pyroides* woodland.

From 1995 to 1998 we were directly involved in fund-raising and facilitating the expansion of the park from 6,500 ha to 28,000 ha (430%). It was home to ten *D.b.b.* in May 2012, before the relocation of two sub-adult females.

3. **Gamma** is situated in the north, about 26,500 ha with two major biomes being savanna and Nama Karoo. Both black and white rhinos (*Ceratotherium simum simum*) in the park use the flat savanna *Acacia mellifera* shrubland. Gamma was home to an estimated 15 *D.b.b.* in May 2012, but in December 2012, 16 were confirmed and possibly 17.

4. **Delta** is an arid area of ± 90,000 ha situated within the Nama Karoo Biome and was home to 12 *D.b.b.* in December 2012.

![Figure 1. Founder populations and present numbers of rhinos in study populations.](image-url)
METHODOLOGY

The necessity for accurately monitoring black rhino populations is not debatable, it is essential for such a valuable endangered species especially during the current poaching crisis.

The data collected in the veldt had to be accurate, reliable and precise, otherwise all future extrapolations would be suspect. The data had to be long-term to provide meaningful information. The major criteria we set for monitoring black rhinos were:

<table>
<thead>
<tr>
<th>Summary of trips:</th>
<th>2002 - 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of field trips:</td>
<td>133</td>
</tr>
<tr>
<td>Trip days (including travelling):</td>
<td>1'234</td>
</tr>
<tr>
<td>Days in the field / veld:</td>
<td>1'002</td>
</tr>
<tr>
<td>Average % population identified per trip:</td>
<td>77.1%</td>
</tr>
<tr>
<td>% Sightings : rhinos undisturbed:</td>
<td>82.8%</td>
</tr>
<tr>
<td>Number of sightings (last 2.5 years):</td>
<td>454</td>
</tr>
<tr>
<td>Rhinos in sight:</td>
<td>1'003 hours</td>
</tr>
<tr>
<td>Sightings with more than one group:</td>
<td>27.1%</td>
</tr>
<tr>
<td>(Group denotes one single rhino or cow with calf)</td>
<td></td>
</tr>
<tr>
<td>Average hours / day in the field (11 - 16 depending on the season):</td>
<td>13 hours</td>
</tr>
<tr>
<td>Maximum number of hours with rhinos in sight on a trip:</td>
<td>24.5 hours</td>
</tr>
<tr>
<td>Tracked on foot in km (last 2.5 years):</td>
<td>922 km</td>
</tr>
<tr>
<td>Average km on foot per trip:</td>
<td>28.8 km</td>
</tr>
<tr>
<td>Maximum km on foot during a trip:</td>
<td>73 km</td>
</tr>
<tr>
<td>Kilometers in vehicle in study areas (last 2.5 years):</td>
<td>23'138 km</td>
</tr>
<tr>
<td>Total km for trips (2.5 years):</td>
<td>91'044 km</td>
</tr>
<tr>
<td>Estimate of total km travelled in 10½ years:</td>
<td>315'000 km</td>
</tr>
<tr>
<td>Digital photographs taken</td>
<td>80'956</td>
</tr>
<tr>
<td>Video footage taken</td>
<td>104 hours</td>
</tr>
</tbody>
</table>

The labour intensive, physical monitoring was not delegated, but carried out exclusively by the authors over the full ten year period. Personal goals were set: to positively identify 75% of rhinos on each trip; to ensure we remained undetected by the rhinos in 80% of sightings; and to attempt to see 100% of all rhinos once a year. Every effort was made to be unobtrusive, which resulted in studying undisturbed, relaxed rhinos and observing natural behaviour. We felt it was unethical to stress the rhinos unnecessarily. Every animal was known to us and individually identifiable by a pattern of ear-notches.

Table 1. Summary of various details of trips in ten years monitoring black rhino

A very successful tactic was to climb to vantage points, find rhinos up to 4 km away, and then plan our approach on foot without disturbing the rhino, getting as close as 20 metres. We often worked separately on any given day to double the coverage. This increased the danger significantly as it meant hiking alone with the ever present possibility of bumping into rhinos, buffalo or lion, which did happen.

Planning played a vital role, and each day key objectives for the trip were set. Trips were undertaken every month, and seven to nine days were spent in the velden every month.

Essential equipment included Kowa scopes (20 - 60x), Canon EOS digital with 25,600 ISO (for night shooting), Sony video, Zeiss binoculars, Leica range finder and Kestrel weather station. Telemetry was used whenever possible; VHF transmitters and GPS satellite foot collars. Two Ford Ranger 4x4 vehicles were essential in the rough terrain.

SANParks have simplified monitoring by giving names to the rhinos. This makes it easier to communicate with field rangers, and we continued the practice.
RESULTS

Overall Growth Rates of the BRMP meta-population

The key to the future of specific rhino populations is encapsulated in one single number - the growth rate - which is determined by a number of reproductive indicators. The growth number can be an oversimplification, and can be deceptive particularly in small populations, but mitigating biases due to small numbers in this study, is the fact that the populations are 100% known. Negative events occurred in all the sub-populations. The numbers of rhino are small, and there is acute awareness of how easily percentages could be exaggerated. However, the study focused on *Diceros bicornis bicornis* in South Africa where the total numbers are inherently very small. In December 2010, South Africa was home to only 171 *D.b.b.*, and only 100 in SANParks\(^1\). At this time, there were 56 rhinos in our study areas representing 56% of *D.b.b.* under SANParks management, and 32.7% of the entire South African meta-population. The numbers in this study may be small, but they form a significant percentage of the subspecies in South Africa.

The growth of 13.4% for the meta-population is excellent, even though it includes many negative biases, different introduction dates in each park, small founder populations and the introduction of immature animals.

*Figure 2: Growth rates – each area*

The lowest growth was recorded in Beta at 9.3% which is still far above average for black rhinos. At the upper end, we note that Bradley Fike\(^{13}\) recorded an overall rate of 10%, while Benson Okita-Ouma\(^{14}\) recorded growths of 5.5% to 11.5 % in the various areas. The BRMP growth of 13.4% is at the higher level recorded for black rhino. Alpha, with the most rhinos, over the longest period of time, showed the highest growth of the four study areas, at 15.1% over 14 years.

However, a far better gauge of growth for this project is to use the last 6 years (2007-2012) as illustrated in Figure 3 when all four parks were included in the study.

*Figure 3: Growth Rates over different periods*

This meta-population growth of 16.5% is not only more representative of the performance, but is consistent whether taking the last 3, 4, 5 or 6 years. A literature search has not revealed other populations with this level of sustained growth over 6 years.
Individual sub-population growth rates

Alpha % Growth Rates

<table>
<thead>
<tr>
<th></th>
<th>6 years</th>
<th>5 years</th>
<th>4 years</th>
<th>3 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007-2012</td>
<td>19.0</td>
<td>18.2</td>
<td>19.6</td>
<td>18.8</td>
</tr>
</tbody>
</table>

Alpha, with a growth of 15.1% for 14 years reflects an even higher and consistent growth average of 19.0% over the last 6 years. The growth for 14 years is markedly lower at 15.1% due to incidents in 2005/2006 which resulted in six deaths after introductions.

**Figure 4. Alpha - excellent performance maintained over time**

In order to evaluate the influence of the incidents during which one newly introduced bull killed six rhinos (including three females), growth for this period i.e. 2005–2008 was only 7.7%. This clearly shows the impact of this single catastrophic event which resulted in the death of 31.6% of the population.

For the full implication, we need to consider that the adult female killed (Blom) had an excellent breeding record. She would have produced another three calves by 2012. The sub-adult female (Ntombi) would also have produced two calves. Therefore, potentially another five rhinos were lost. This population would have had 45 rhinos instead of 34 - this amounts to +32.4% or 11 more rhino. The overall growth would change from 15.1% to 17.3%.

Other negative factors for this area were a very small founder population (five), the unnecessary death of a female calf due to human interference in 2008, and the injury to the only breeding bull during immobilization which had a temporary negative impact by delaying conception in a few rhinos.

**B. Beta** (population introduced in March/April 2002 – Figure 5).

Beta showed a marked slow-down from 18.1% to 8.3%.

The major negative factors were again a small founder group (five), with a male skewed sex ratio (0.67♀ : 1♂) and the fact that the only mature bull had to be removed within the first year as he continually attacked the older female cow. In the first week of July 2005, the first calf in this area died due to human interference and the second cow, Dundi, only produced her first calf at 12½ years - no apparent reason.

The subsequent precipitous fall in growth from 18% to 8% was due to a high tourist impact, interference from people eradicating alien species, and the building of major tourist roads into the previously exclusive areas inhabited by the rhinos. Also, carrying capacity had been exceeded. On the positive side, six of the seven calves born were female.

**Figure 5. Declining growth, indicative of problems**

**C. Gamma** (entire population relocated to new park in late 2006 – Figure 6)
This area also shows a slow down from 16.1% to 12.4%

A marked slowdown from 19.1% (2007 to 2010) to 12.4% (2010 to 2012) was due to the carrying capacity being exceeded. As opposed to the Beta area, mainly male calves have been born here. Of the ten calves born since introduction to Gamma, seven have been male. A clear indication of the stresses can be seen in the widespread destruction of *Acacia tortilis* trees.

![Figure 6. Signs of problems](image)

**D. Delta** (first *D.b.b.* introduced in 2005 – Figure 7)

Growth has moved very positively from 9.9% to 19.8%. Introductions took place in 2005, 2007 and 2008 without any breeding. In order to calculate growths, we simply took the founder population as seven in 2006. This had the negative impact of zero growths from 2007 to 2009. All the females were immature. Again, a very small founder population introduced over four years, and in 2007, the population was skewed to males (0.25♀: 1♂). The positives for this area are that it has a high carrying capacity, and that breeding has now started in earnest.

![Figure 7. Excellent progress once breeding started](image)

**Reproductive Indicators**

<table>
<thead>
<tr>
<th>Parameters in blue</th>
<th>Very Poor to Poor</th>
<th>Poor to Moderate</th>
<th>Moderate to Good</th>
<th>Good to Excellent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage Growth:</td>
<td>&lt; 2.5%</td>
<td>2.5% - 5.0%</td>
<td>5.0% - 7.5%</td>
<td>&gt; 7.5%</td>
</tr>
<tr>
<td>BRMP Meta-population:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inter-calving Interval:</td>
<td>&gt; 3.5 yr</td>
<td>3.5 - 3.0 yr</td>
<td>3.0 - 2.5 yr</td>
<td>&lt; 2.4 yr (30 m)</td>
</tr>
<tr>
<td>BRMP Meta-population:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Cows calving / year:</td>
<td>&lt; 29%</td>
<td>29% - 33%</td>
<td>33% - 40%</td>
<td>&gt; 40%</td>
</tr>
<tr>
<td>BRMP Meta-population:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average age at 1st calf:</td>
<td>&gt; 7.5 yr</td>
<td>7.5 y - 7.0 yr</td>
<td>7.0 y - 6.5 yr</td>
<td>&lt; 6.5 y (78 m)</td>
</tr>
<tr>
<td>BRMP Meta-population:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion calves 0 to 12 mths:</td>
<td>&gt; 8.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRMP Meta-population:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality Rate:</td>
<td></td>
<td></td>
<td></td>
<td>4% or less</td>
</tr>
<tr>
<td>BRMP Meta-population:</td>
<td></td>
<td></td>
<td></td>
<td>2.6%</td>
</tr>
</tbody>
</table>

We have recorded and analysed all the reproductive indicators listed in the literature by Raoul du Toit\textsuperscript{15} and Mike Knight.\textsuperscript{16}

We used Du Toit’s parameters to rate the BRMP meta-population in May 2012.

**Table 2. Comparing the BRMP meta-population study with R. du Toit’s parameters**
Comments on reproductive indicators

With the excellent growth rate recorded, it would follow that most parameters if not all, would be scored as “good to excellent”. This was the case, with the surprising exception of age at first calf. Of 21 mothers, only six had their first calf at ages younger than 6.5 years. Fike\textsuperscript{10} recorded 80 months as the average age at first calf, to our 83.7 months (Table 2), while Okita-Ouma\textsuperscript{11} recorded 5.5 years (66 months) and 6.5 years (78 months) in two populations.

Other Parameters:

Sex ratios

After the relocations (see page 12) in May 2012 and April 2013, sex ratios improved as follows:

Table 3. Sex ratios $\frac{♀}{♂}$ improved after relocations in May 2012 and April 2013

All populations now show a favourable sex ratio.

BRMP Meta-population Age Structure

Figure 9. Percentage Age Structure for this Study in May 2012
Mortality

Although mortality for the meta-population, at 2.6%, is lower than either the 3% (Knight \textsuperscript{16}) or 4% (Du Toit \textsuperscript{15}) previously suggested, it is important to analyse these mortalities. Of a total of eleven deaths, only two (18%) were from natural causes (fighting in both cases). Significantly, although we recorded 56 births, no calves died from natural causes, but two calves (4 – 6 months) died after human disturbance.

![Figure 10. Analysis of deaths](image)

![Figure 11. Man-induced deaths analyzed.](image)

Reproductive Indicators for each sub-population:

<table>
<thead>
<tr>
<th>Sub-population</th>
<th>Alpha</th>
<th>Beta</th>
<th>Gamma</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Rate;</td>
<td>15.1%</td>
<td>9.3%</td>
<td>12.0%</td>
<td>9.9%</td>
</tr>
<tr>
<td>ICI;</td>
<td>25.3 m</td>
<td>32.4 m</td>
<td>28.3 m</td>
<td>24.0 m</td>
</tr>
<tr>
<td>% Cows calving / year;</td>
<td>50.4%</td>
<td>35.0%</td>
<td>44.0%</td>
<td>55.6%</td>
</tr>
<tr>
<td>Average Age at 1st calf:</td>
<td>79.7 m</td>
<td>78.0 m</td>
<td>94.3 m</td>
<td>82.5 m</td>
</tr>
<tr>
<td>Proportion of calves: 0 - 12 m:</td>
<td>17.6%</td>
<td>20.0%</td>
<td>12.5%</td>
<td>25.0%</td>
</tr>
<tr>
<td>Mortality Rate:</td>
<td>3.4%</td>
<td>2.0%</td>
<td>3.1%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Sex Ratio F : M</td>
<td>1.36 : 1</td>
<td>2.33 : 1</td>
<td>0.78 : 1</td>
<td>1 : 1</td>
</tr>
</tbody>
</table>

The best reproductive indicators are in Alpha and Delta, mainly due to very little tourist impact in the former, and a very large area in the latter.

Table 4. A comparison of the four sub-populations’ reproductive indicators
**Body Condition:** Each rhino’s body condition is scored at every sighting by both authors independently, and the table below presents average individual body condition score over the period of the study. Results indicate no variability between seasons, and the scores are surprisingly consistent - the average for the meta-population was 4.41 out of 5. These body condition scores are a very good indicator of the suitability of the habitat in all areas.

<table>
<thead>
<tr>
<th></th>
<th>Alpha</th>
<th>Beta</th>
<th>Gamma</th>
<th>Delta</th>
<th>Meta-population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range:</td>
<td>3.7 to 4.7</td>
<td>4.2 to 4.5</td>
<td>3.8 to 4.5</td>
<td>4.2 to 4.5</td>
<td>3.7 to 4.7</td>
</tr>
<tr>
<td>Average:</td>
<td>4.5</td>
<td>4.4</td>
<td>4.4</td>
<td>4.4</td>
<td>4.4</td>
</tr>
</tbody>
</table>

*Table 5. Body condition*

**Inter-calving Intervals (ICI)**

Alpha 8 cows with ICIs and 10 with calves.
Beta 2 cows with ICIs
Gamma 4 cows with ICIs
Delta 1 cow with ICI and 4 cows with calves

*Figure 12: ICIs of sub-populations*

**The most productive cows:** Ubhejane gave birth to calf # 13 at age 36 years

<table>
<thead>
<tr>
<th>Cow</th>
<th>Number of calves</th>
<th>♀</th>
<th>♂</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ubhejane</td>
<td>13</td>
<td>2</td>
<td>11</td>
<td>Gamma</td>
</tr>
<tr>
<td>Blom</td>
<td>11</td>
<td>10</td>
<td>1</td>
<td>Alpha</td>
</tr>
<tr>
<td>Faru</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>Beta</td>
</tr>
<tr>
<td>Nkombe</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>Gamma</td>
</tr>
<tr>
<td>Shibula</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>Alpha</td>
</tr>
</tbody>
</table>

*Table 6: The most productive cows in the BRMP meta-population*
Percentage of cows calving per year

<table>
<thead>
<tr>
<th>Percentage of cows calving per year</th>
<th>50.4%</th>
<th>35.0%</th>
<th>44.0%</th>
<th>55.6%</th>
<th>47.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>1999-2012</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta</td>
<td>2002-2012</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma</td>
<td>2006-2012</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td>2010-2012</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meta-pop</td>
<td>1999-2012</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 13. Percentage of Adult cows calving per year**

**Figure 14. Average of 47.0% of adult cows calving /year.**

**Gestation period**

We have one accurate calculation of a black rhino cow’s gestation period, i.e. not less than 465 days or 15.30 months, and not more than 475 days or 15.58 months. (Bertschinger 1994 states 15.4 months).
GENEAOLOGY

Shibula. The cow originally from Etosha, via Lisbon Zoo and finally to Alpha has given birth to eight calves known and seen, and a ninth was expected later in 2012.

Ubhejane. A founder introduced in August 1987, from Namibia and translocated to Gamma in September 2006, has given birth to thirteen calves. The last calf was born when she was 36 years old.

©A. Mavromandis & Sue Dowié
Developed Genealogy over 11 years of Black Rhino Monitoring Project, with acknowledgement and thanks to Dr. Anthony Hall-Martin and Dr. Guy Castley for historic data.
Population Dynamics, Interventions Recommended

Interventions:

Interventions have inherent risks when immobilizing, transporting and releasing rhino, and can lead to additional problems. Therefore, it is our view that populations do best with the least interference. However, the regular ear-notching exercise does assist in positively identifying each rhino. We also believe that stable family units contribute to better growth rates, and that black rhinos have a complex social group structure which should remain intact if at all possible.

Three of the sub-populations needed assistance to ensure long term optimal growth, and it was decided that if carefully thought through and planned meticulously, the interventions would be beneficial at minimal risk. As usual, we committed ourselves to at least six weeks of post-release intensive monitoring of any translocations.

Warning signs in specific areas:

In our Population Dynamics report (October 2011), we commented on the prevailing population growth rates, and how positive the situation appeared, however, there were numerous early warning signs, which in our judgment, needed to be addressed urgently.

Beta

Excellent sex ratio (3♀:1♂), but the ecological carrying capacity (ECC) should not be more than ten to twelve rhinos. The ICI of the two cows increased from 24 and 26 months to 35 and 37 months. Our estimate of ECC agreed with that of Castley. For Beta there were too many rhinos and too many females.

Recommendation: Remove two five-year-old sub-adult females, which would reduce the total number of rhinos and marginally reduce the female sex bias.

Gamma

Sex ratio skewed to male (0.6♀:1♂). We estimated the ECC at 12-15 rhinos. Castley used 0.055 per km² for the old park - this would give a maximum of eleven black rhinos. At the AfRSG meeting in Gamma in March 2010, it was suggested that the tree damage could be an early warning sign. Once again, the problem was too many rhinos, and in this case, too many males.

Recommendation: Remove eight sub-adults males, over two years, reducing the total population and improving the sex ratio. Move young males to a bull camp.

Delta

This is a large area, with too few rhino. To have a viable breeding group, this area needed more females.

Recommendation: Move the two sub-adult females from Beta to Delta.

Population Dynamics Conclusion

SANParks arrived at a different solution which did not address the problems of Beta and Delta. Our recommendations were finally accepted and carried out in May 2012, and April 2013. The short-term problems had been addressed, but the long-term issues remained – in essence more rhino habitat is needed.
Since 1991, we have been involved in 16 translocations with SANParks – all documented, photographed and videoed. We gave hands-on direct assistance, recorded measurements, facilitated sponsorship by DSWF and the authors personally for transmitters (VHF and GPS satellite foot collars), as well as transport for six rhinos from Namibia.

Although a total of 41 rhinos were translocated, none died during the 16 translocations, however there were several post-release problems (60%) and deaths.

Table 7. Translocation and release indicators.

It was obvious that intensive post-release monitoring was essential after the death of a three-year-old female, and we volunteered our services and included this in the project. The success or failure of a translocation is not only the physical capture and transportation of the rhinos, but also to ensure they survive and adapt in the new areas, and resident rhinos are not wounded or killed in the months after the translocation. Very clear guidelines are given in the IUCN publication edited by Emslie et al, but in our experience, not always followed. Any translocation whether long or short should be properly and professionally planned and executed and should not be considered as a fun or recreational outing, no matter how many times it has been done before.

This should apply at captures as well. Unanticipated problems do occur, such as a cow suddenly standing up before the antidote was given. She had to be darted twice before being re-captured. Three months later she gave birth to her second calf. This was also the second time that the same cow had been translocated when heavily pregnant. Most problems could be avoided if the capture team communicated with rhino monitors/researchers prior to capture, and sensitive, intensive post release monitoring was carried out after every translocation.

Re-wilding of two tame black rhino

Shibula’s journey

This was a complicated journey in 1991, from Lisbon Zoo, Portugal, to a national park in the north of South Africa, and involved travel by sea (8,560 km), air (820 km) and army truck (130 km). We were directly involved from the beginning. The journey was sponsored by David Shepherd and our pharmaceutical company Lagamed. The journey was documented by the environmental TV programme 50/50, and other partners in this amazing conservation story were SANParks, DSWF, Lisbon Zoo, South African Air Force and Army.

We travelled to Lisbon with the TV crew, the park warden and a vet to ensure a smooth transition from a tame zoo rhino to a wild rhino. She was trained to walk into her crate to find food, and her keeper and the vet accompanied her on the 13-day voyage to Cape Town. Shibula’s arrival at the docks and transfer to the Air Force base with a traffic police escort, created much excitement and publicity. Her crate was loaded into a C130 Hercules aircraft
for the flight north to a town near the national park. We accompanied her on the flight and the final 130 km by road to her new home. At midnight, she slowly walked out of the confinement of the crate into a boma with African soil underfoot. We all slept alongside her boma.

Shibula spent about 3½ months in the boma, acclimatizing to the new diet and safely meeting the resident rhino. The gate was opened about an hour before sunrise, with only five of us sitting on top of her old crate in total silence. It was over an hour before she slowly walked the few steps outside the boma. She was released into a 1,000 ha camp. Shibula was so tame you could entice her to the vehicle by cutting an apple in half. She gave birth to her first calf on 16 September 1994, a female we called Dundagos, which means “we have achieved” in the Nama language.

In the 22 years that Shibula has been in the wild she has given birth to eight calves that we know of and a ninth was expected in 2012. Two of her calves have had three calves each. The goal of her translocation was to increase the small founder population and improve the growth rate of D.b.b. in South Africa – which she did by another 15 black rhinos, eight of which are females. The forethought, planning, care and time devoted to her translocations resulted in a successful outcome. Thanks to Dr. Anthony Hall-Martin.

**Thandi and Kapela – two hand-raised black rhinos**

Thandi (D.b.b.) and Kapela (D.b. minor) were both born in bomas and were cared for at a rehabilitation facility. We travelled to Pretoria to see both baby rhinos every month to monitor their progress and photograph their growth and development, which has helped us to age calves in the veld. They had continual diarrhoea, from an almost exclusive diet of milk formula and juice. From our observations, calves start browsing with their mother’s supervision at two months of age.

At eight and nine months of age, Thandi and Kapela were moved together to a boma in a national park. After 13 months, they were released into a 400 ha enclosure with limited tourist access. Unfortunately, private lodges had access and bad behaviour resulted in Kapela taking an intense dislike to vehicles, which has compromised his re-wilding in a private reserve. Thandi at 5½ years was relocated to Delta and released with a younger female (~ 3 years old), into bomas for three weeks. Thereafter, we prevailed in providing a temporary release camp, far from tourists, and we intensively monitored their final release from the bush camp for about six weeks, with daily monitoring by one of us.

Three days after release and on a very hot day (38°C) during which we established they had not drunk water for at least 24 hours, we found both young rhinos on top of a very steep treeless hill. Previously, they had only had water from a concrete reservoir. Together with two field rangers we took 60 litres of water and two troughs to the rhinos, and then persuaded them to follow Sue down a less difficult slope. It was an intensely moving experience, and the intervention was essential in the circumstances. Subsequently, they learned to drink in pools of water in the rivers, met the resident bull and both have had calves. A very long journey, and ultimately a successful re-wilding of a rhino hand-reared from birth.

We estimate that the 17 rhinos produced by Shibula, her calves and Thandi account for approximately 8.5% of all D.b.b. in South Africa. Both exercises were very costly in time and money, but very well worth the effort in the long-term.
DISPERSAL

A Rhino camp 4,540 ha  
B Rhino camp 3,179 ha  
A + B Total available to rhinos 2005 to 2007 = 7,719 ha

Map # 1: Rhino camps in Alpha showing dispersal

Over ten years, we followed up on ten of fourteen translocations (71%). One release (7%) in 1991, was before the project started; another two we were only able to monitor intermittently. We found one old bull dead a few days after an ill-conceived release in an inappropriate area. All observations have been plotted on 1:50,000 topographical map.

After translocations where either all rhinos were known to each other, or a great deal of effort and care was taken (releasing far from resident rhinos, erecting a temporary holding camps), together with intensive post-release monitoring, the rhinos established themselves successfully. In established populations, dispersal has taken one to two years after fences were removed. (See map).

Some dispersals were forced when two young bulls were introduced to two separate adjacent areas with an inadequate fence separating the camps. Sadly, six rhinos died (including three females). Once the offending bull was relocated and the fences removed, dispersal continued at a steady pace.

Pregnant cows often moved to different, less populated areas to have calves and then remained in the new range. In Delta, within days after introduction a young bull went on a 30 km walk over a weekend, lost body condition by the time he returned, but then he stayed in the vicinity. A young female walked 41 km in 16 hours, spent a small percentage of that time along a boundary fence, and when she returned to the starting point also settled in the area with a bull for some time. She moved into the hills when she was ready to calve.

In Gamma with ± 26,500 ha, the black rhinos utilised only about 5,500 ha. When the breeding bull died, the oldest of the sub-adult males was seven years old and pushed two other young males to the outskirts into marginal habitat.

BEHAVIOUR

Studying behaviour was not an original objective of this project, but followed naturally due to the time spent in proximity to rhinos without being detected. The behaviour discussed may be viewed as anecdotal as it was not a formal scientific ethology study – more a behavioural field study. The observations have ecological validity and are based on over 1,300 hours of studying natural behaviour of undisturbed rhinos, while the interpretations are our own personal opinion.

The black rhino is characterized as having poor eye-sight, being aggressive and solitary. Our opinion differs markedly. Rhinos have superb motion detection\(^1\), and the slightest movement focuses their attention immediately and accurately. In over ten years of tracking
and walking with rhinos, with many unexpected close encounters, we have never been aggressively charged, even by mothers with calves. They assess the threat, may mock charge to get the object to move and so identify it. Once the threat has been assessed, the rhino will walk away or run a short distance and turn back, always with head held high. Serious charges are normally silent with head down, which we have witnessed from a distance.

Although in biological terms solitary means not living in herds, and this is accurate for black rhino, they do have a complex social system. In 27.1% of our sightings involved three or more, and sometimes up to ten black rhinos, sometimes within 30 m of each other, close together interacting.

Social interactions involved nose-to-nose contact (greeting), and sub-adult related or unrelated females acting as surrogate mothers by engaging the calf while the mother browsed, which helped a lactating cow improve condition quicker. If rhinos are too close and not welcome, a growl will suffice to move them apart. The length of a greeting and co-mingling can indicate individual likes and dislikes between rhinos. Black rhinos have a wide range of vocalizations, from high pitched squeak between a cow and her calf, to squealing, snorting, growling and even roaring. Rhinos appear to “visit” a mother with a new calf, thereby introducing it to the local population. Mothers encourage their calves to browse beginning at just 2 months of age, and this is important due to the high number of orphans resulting from poaching incidents. Hand-raised calves should have access to browse to prevent diarrhea from too much milk formula. Calves run behind their mother in times of stress, but when safe and relaxed they often run in front.

“The main function of Flehmen is to transfer air containing pheromones and other scents to the vomeronasal organ, a chemosensory organ.” This explains our observations of all age groups, both male and female, displaying the Flehmen response, even calves in response to their mother’s urine.

Three matings were observed out in the open during the day. In 2008, we observed the mating process over four hours, a mere 102 m from us. After the bull attempted to mount the cow for the tenth time, he rested his head on her rump in total exhaustion. In another observation, the 19 month old calf stayed close to its mother and not more than 30 m from the mating adults. All were completely relaxed. Successful bulls that have sired many calves have exhibited caution and patience when approaching females and often waited for the female to initiate contact.

We have regularly observed cows using their horns to break higher branches to get to the new shoots for their calves and themselves. They place the branch between the two horns and lever it down or use their chins to push down the branch. Not only do rhinos use rubbing rocks, but they often lick the rock afterwards – possibly licking blood from crushed ticks. Rhinos sleep when tired day or night. Sub-adults appear to sleep longer without moving, whereas cows get up regularly to suckle young calves. However, a mother can sleep for five hours before standing up, as a very small calf can suckle while the mother is lying down. Mothers also often change position every one to two hours. We have often observed what can only be described as “playful” activity, mostly among calves and sub-adults, but on a few occasions adults have joined this activity of chasing each other around bushes and suddenly changing direction.

Black rhinos often move high up on hills or ridges and we believe that in summer, it is for the cool winds, and in winter, for warmth due to a temperature inversion and better browse.
The above summary of behaviour recorded was observed regularly and the patterns repeated by different rhinos in different areas and we believe the activities to be natural black rhino behaviour, which can be attributed to the general population.

SECURITY

Rhino poaching in South Africa continues unabated.

* Official figures to 07 August 2013 indicate 553 dead rhinos.23 A conservative extrapolation means over 900 rhinos will be killed in 2013.

Figure 15. Rhinos killed in South Africa from 2009, to an estimate for 2013

Kruger has deployed unmanned drones, two surveillance aircraft, the army, and are using tracker dogs, but the carnage continues at ever higher levels. In order to accurately assess the presence of poachers and extent of the killing, intelligence starts with effective monitoring and knowledge of every population.

In mid-2010, we realized we were as guilty as everyone else - expressing outrage and merely counting the numbers killed. One rhino poached in our areas would not simply be a number, but a known individual. SANParks and the Government were putting all their resources into Kruger, while leaving the smaller parks vulnerable and chronically short on ranger density per hectare, as well as equipment. In one study area, ranger density was at 22% of levels suggested by Du Toit.15 We identified the threats in the four project areas and developed security plans according to the specific threats.

Security Plans

By October 2010, we had viable security plans for each park proposed by the section rangers and ourselves. Approval was granted from SANParks head office. Funding would be sponsored by the David Shepherd Wildlife Foundation and the authors personally.

To date funding has covered items such as: overtime payments for existing field rangers, employment of new field rangers, basic and advanced anti-poaching courses and fire arm courses, and 3) new contract staff (!Khu Bushmen trackers) specifically for rhino protection. In addition the funding has allowed the purchase of five Kawasaki Mule 610 all terrain vehicles to ensure mobility of field rangers, basic equipment such as two-way radios, binoculars, cameras and spotting scopes, material for a bush camp, ranger personnel kits, sponsored private pilot’s license for two senior staff, construction of look-out platforms, signs and CCTV cameras on the perimeter fences, electric access gates and spot lights, a Wendy house Observation Post and overnight accommodation and a fully equipped Ford Ranger 4x4 Pick-up.

Costs: Since October 2010, total costs have been R1,706,000 (US$ 170,600) of which R1 million sponsored by DSWF and the balance by the authors.

Results

No rhinos have been lost, regardless of poaching activities close to the BRMP areas. Motivation of the field rangers increased. Sadly, we believe that there is not nearly a powerful enough collective will to save rhinos from extinction. It is simply about money.
Cagan Sekercioglu, ornithologist and professor at the University of Utah who runs an award-winning conservation group in his native Turkey says it all; “...the government talks about conservation, but its priority is to convert nature into cash” (National Geographic interview, May 2013).

FORECAST (2012 - 2022) : Number of black rhinos in four project areas

<table>
<thead>
<tr>
<th>Last 3 years Growth Rate 2010 - 2012</th>
<th>Forecast Growth Rate 2013 - 2022</th>
<th>Final estimate of numbers 2012</th>
<th>Forecast numbers 2022</th>
<th>Relocate</th>
<th>Population after relocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>+18.8%</td>
<td>+11.7%</td>
<td>38</td>
<td>114</td>
<td>-19</td>
</tr>
<tr>
<td>Beta</td>
<td>+8.3%</td>
<td>+9.9%</td>
<td>7</td>
<td>17</td>
<td>-7</td>
</tr>
<tr>
<td>Gamma</td>
<td>+12.4%</td>
<td>+8.8%</td>
<td>17</td>
<td>39</td>
<td>-27</td>
</tr>
<tr>
<td>Delta</td>
<td>+19.8%</td>
<td>+13.4%</td>
<td>14</td>
<td>49</td>
<td>-6</td>
</tr>
<tr>
<td>Meta-pop</td>
<td>+16.5%</td>
<td>+11.1%</td>
<td>76</td>
<td>219</td>
<td>-59</td>
</tr>
</tbody>
</table>

Table 8: Forecast for each sub-population of the number of rhinos in ten years time

Assumptions:
Conservative future growth of 11% (at present 16%). Forecasts done per individual animal, with longer ICI’s than at present. Births at +7 years old for individual female rhinos. New calves - assumed 50% female. Mortality of 2% per year. Cows older than 36 years excluded.

Results:
The four sub-populations of 76 animals will increase to 219 by 2022. To keep populations at optimum growth, we will have to relocate 59 rhinos or 27% of the population. This will leave 160 rhinos in the four areas.

Future Problem:
The habitats and the breeding populations are excellent. But the carrying capacities are limited in all four parks when projecting to 2020 or 2022. If South Africa is to reach the targets set by the BMP, it is critical that more national parks are prepared to receive black rhinos, and more private reserves need to agree to a custodianship arrangement. Expansion of the parks in the project areas is another way forward, i.e. acquisition of land. A Development Plan linking Graaff-Reinet to Cradock is an example of an initiative that should be pursued as a priority.

Time Frame
As the purchase of land and expansion of national parks is a long term endeavour, these initiatives should start immediately. The project organized by the authors to expand Beta took ten years (1996 to 2006) taking it from 6,500 ha to 28,000 ha. Even preparing existing parks to receive black rhinos can take three to four years.

BMP TARGETS
Biodiversity Management Plan for the black rhinoceros in South Africa 2011–2020 requires:

In the short term (10 year goal - by 2020) a meta-population of 260 D.b.b. In the long term (no date given), a meta-population of 500, with one population of at least 100, and another with at least 50. An average growth rate of at least 5%.
The BRMP’s accomplishments towards the BMP Targets

- One population can be maintained at 90-100 rhinos, and a second population can be kept at 40-50 rhinos, which confirms the BMP target can be accomplished.
- The present growth rate of 16% for the BRMP meta-population, and a future growth reliably predicted at a minimum of 11%, will achieve the BMP minimum of 5%.
- In 2010, the BRMP areas made up 32.7% of all *D.b.b.* numbers in South Africa. If this ratio is maintained, then the 219 rhinos in the four areas will make up 33% of the total South African meta-population in 2022, giving a total of 670 *D.b.b.* (BMP target of 500 rhino), or 134% of the long term target.
- The four study areas will have 179 rhinos or 69% of the short term BMP target of 260 by 2020. If we assume this contribution to the meta-population is maintained at 33%, the total rhinos will be 542 (double the BMP target of 260).

Black rhinos are not yet the major target of poachers. The biggest threat to achieving the targets for *D.b.b.* is the present lack of additional land or areas to which excess animals from the study area can be relocated. This is not stressed sufficiently in the BMP and it is our belief that the acquisition of additional habitat / land should be at the core of the BMP. This becomes even more essential if the BMP recommendation in section 5.1.2.2 is to be followed, i.e. “do not stock with more than 40% of the estimated capacity for the area in question”.

**TERMINATION of the Project in three of four areas**

A large part of this project, which started in 1991 with the repatriation of Shibula from Lisbon Zoo, was abruptly terminated by the SANParks regional manager in charge of three of the four areas on 16^th^ May 2012, due to a dispute involving the computer access codes for GPS satellite foot collars. Senior directors and the Chairman of the AfRSG met with us in an attempt to reconcile disparate views, but the regional manager refused to meet with us. We were all unable to convince him that the dispute should be resolved in the best interests of the rhinos. We continue to work in one research area, providing monitoring and financial support ourselves. A 4x4 vehicle was donated in April 2013, and total security costs will amount to almost R300’000 for this financial year.

**CONCLUSION and RECOMMENDATIONS**

This ten-year study by external researchers with international and local funding has demonstrated that the four sub-populations of *D.b.b.*, given good habitat and limited human interference, can breed at a rate of 15% over 14 years, and will contribute significantly to the survival of the sub-species in South Africa by exceeding the BMP targets and growth rate of 5%. However, is there the will to make the effort required to make more land available, improve the levels of monitoring and do what is best for the rhinos and not what is politically expedient?

Factors critical to continuing this success, are:

- Careful and unobtrusive monitoring is essential to ensure reproductive indicators remain positive. Interventions should be undertaken only when necessary.
- Black rhinos breed best where the tourist impact is kept to a minimum. Areas should be dedicated for breeding black rhinos with limited tourist access, and one or two areas specifically for “surplus” bulls where tourists can see black rhinos.
- **Beta** is an example of how high tourist density and obtrusive monitoring impacted negatively on the animals. One month after the abrupt termination of the project, a
field ranger was severely injured and nearly killed by a cow which had been separated from her calf. The calf later died. We recommend that Beta should not be a breeding area, but rather a camp for “surplus” bulls, which would satisfy tourists.

- Long-term investment and partnership with the private sector can play a pivotal role in developing rhino areas and monitoring to provide independent analysis of progress and performance.

- The forecast of these populations indicates it is imperative to find new areas for the surplus animals, and to acquire more suitable habitat for black rhino. This is the single most important component to ensure the future of the species.

- The study also validates the value of a long-term investment to rehabilitate rhinos. In this study, two rehabilitated females resulted in 18 additional rhinos.

- Formal ethology studies should be pursued to better understand black rhinos, but such observations should be done in an unobtrusive manner, which our study proves is possible.

- Above all, a dedicated senior rhino manager or coordinator within SANParks is vital for the future of rhinos in national parks.

- Security plans for all areas with rhinos.

- Respect for dedicated external researchers working closely and sharing information with SANParks is essential.

ACKNOWLEDGEMENTS

David Shepherd: for his generosity in donating paintings. The David Shepherd Wildlife Foundation and former CEO Melanie Shepherd, for support and funding the monitoring and security projects. Eastern Cape Motors and Greg Williamson, for keeping our vehicle safe in between monitoring trips at no charge. Other donors: De Villiers Family Trust, JD Group of Companies, JMD Trust, Peter & Elsabeth Metz, Melanie Adendorff for caring for Thandi, Kapela and Danny in the bomas. John Adendorff for coming to the rescue of Ntombi and others. All the Field Rangers including: Alec Nomvulo, Johnson April, Piet Booysens, Pokkie Benade, Chocolate Bosch, Donovan Antonie, Surprise Mlambo, Robert Rikhotso, Richard Ockers from whom we learnt about walking in the veld and the terrain, and who have worked long hours and showed incredible interest in our project. !Khu Bushmen: Marteu Tami, Augustu Chamba, Tiber Likongo, who have the most remarkable tracking skills, and an amazing work ethic, we worked together in the veld with mutual respect. We salute them.

Dr. Anthony Hall-Martin: senior researcher for this project. Dr. Guy Castley: first to see the potential of our monitoring contribution. Dr. Hector Magome, Dr. Mike Knight, Dr. Hugo Bezuidenhout, Dr. Markus Hofmeyr, Dr. David Zimmermann, Dries Engelbrecht, Dr. Peter Bradshaw, Norman Johnson, Deon Joubert, Pumla Mzazi, Mzwandile Mjadu, Riaan Nel, Robyn Woods, Johan de Klerk, Nico van der Walt, Abel Ramavhale. Dr. Pete Morkel: keeping contact and always available for advice. Dr. William Fowlds: for assisting Blom.

Besides the rhinos, our greatest motivation came from the field rangers and the Bushmen. We thank them for that and their dedication to the rhinos.
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6. Section Ranger’s Proposal to DSWF for Security Funding


9. Section Ranger’s Proposal to DSWF for Security Funding


11. Park Manager’s Proposal to DSWF for Security Funding

12. Section Ranger’s Proposal to DSWF for Security Funding


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23. Department of Environmental Affairs & Tourism website www.environment.gov.za

**Alpha Population Detailed Growth Rate Analysis**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Founder: 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1999 7</td>
<td>40.0%</td>
<td>(A) Exclude 2 from 2005</td>
<td>(A) Exclude 2 from 2005, plus 1 removal in 2006</td>
</tr>
<tr>
<td>2000 7</td>
<td>0.0%</td>
<td>(B) Totally exclude growth of 2005</td>
<td></td>
</tr>
<tr>
<td>2001 9</td>
<td>28.6%</td>
<td>(C) Change the base of 2004 by adding 2, to calculate growth for 2005.</td>
<td></td>
</tr>
<tr>
<td>2002 9</td>
<td>0.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003 12</td>
<td>33.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004 12</td>
<td>0.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005 15</td>
<td>25.0%</td>
<td>(-2) 13 8.3% * Exclude year 2005</td>
<td>14 15 7.1%</td>
</tr>
<tr>
<td>2006 13</td>
<td>-13.3%</td>
<td></td>
<td>15 13 8.3%</td>
</tr>
<tr>
<td>2007 16</td>
<td>23.1%</td>
<td></td>
<td>(+1) 14 7.7%</td>
</tr>
<tr>
<td>2008 18</td>
<td>12.5%</td>
<td></td>
<td>(-1) 16 14.3%</td>
</tr>
<tr>
<td>2009 22</td>
<td>22.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010 26</td>
<td>18.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011 32</td>
<td>23.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 2012 34</td>
<td>6.3%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 3 Methods of Dealing with 2012

1. Average annual growth for 13.42 years. 16.3% 15.1% 15.6% 15.0% 16.0%

2. Worst case, ignore potential births after May, i.e. 14 years. 15.6% 14.5% 14.9% 14.4% 15.3%

3. Accurate forecasts predicted 4 births June to December 2012. (Growth 18.8% for 2012) 16.5% 15.3% 15.9% 15.3% 16.2%

### Average of all Growths for (A), (B) & (C)

15.11%

**Annexure Y**

**Method of Adjustment for Removal (1 ♂) in 2006, & Introduction (1 ♂) in 2007, plus using the (A) method.**

**NOTES:**


2. Every single rhino is known and accurate forecasts of births was routine, using historic inter-calving intervals.

* 35 Calves were born in the period 1999 to May 2012.

2. Growth rates for the last 6, 5, 4 and 3 years in the Paper are straightforward as they exclude the 2005/2006 introductions and bull relocations of 2006/2007. Calculations can be verified from the year end population numbers, left. As verification of Alpha population growths of 19.0%, 18.2%, 19.6% and 18.8% over 6, 5, 4, and 3 years, if we exclude the partial 2012 year, then the last 5 years growth is accurately 19.8% (+23.1, +12.5, +22.2, +18.2, +23.1).

3. Final assumptions of growth used in the Paper.
   - Used growth of 15.1%, i.e. 13.42 years and method (A) above
   - Correlates with average of all methods (15.1%).
   - Correlates exactly with period of 13 years (excluding 2012), i.e. 15.1%.
   - Ignores the bull removal and introduction of 2006 / 2007

Therefore our average annual growth figure of 15.1% is conservative.
## Beta Population Detailed Growth Rate Analysis:

<table>
<thead>
<tr>
<th>Year</th>
<th>End Year Population Numbers</th>
<th>Yearly Growth</th>
<th>Adjustment due to removal of one  ♂ within a year of introduction, due to serious harrassment of a cow.</th>
<th>For interest sake, add back calf killed by low flying helicopter &amp; her progeny.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Founder 2002: 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>4</td>
<td>-20.0%</td>
<td>Founder: 4</td>
<td>4</td>
</tr>
<tr>
<td>2004</td>
<td>5</td>
<td>25.0%</td>
<td>5 25.0%</td>
<td>5 25.0%</td>
</tr>
<tr>
<td>2005</td>
<td>4</td>
<td>-20.0%</td>
<td>4 -20.0%</td>
<td>5 0.0%</td>
</tr>
<tr>
<td>2006</td>
<td>4</td>
<td>0.0%</td>
<td>4 0.0%</td>
<td>5 0.0%</td>
</tr>
<tr>
<td>2007</td>
<td>6</td>
<td>50.0%</td>
<td>6 50.0%</td>
<td>7 40.0%</td>
</tr>
<tr>
<td>2008</td>
<td>8</td>
<td>0.0%</td>
<td>6 0.0%</td>
<td>7 0.0%</td>
</tr>
<tr>
<td>2009</td>
<td>8</td>
<td>33.3%</td>
<td>8 33.3%</td>
<td>9 28.6%</td>
</tr>
<tr>
<td>2010</td>
<td>8</td>
<td>0.0%</td>
<td>8 0.0%</td>
<td>9 0.0%</td>
</tr>
<tr>
<td>2011</td>
<td>8</td>
<td>0.0%</td>
<td>8 0.0%</td>
<td>10 11.1%</td>
</tr>
<tr>
<td>May 2012</td>
<td>10</td>
<td>25.0%</td>
<td>10 25.0%</td>
<td>12 20.0%</td>
</tr>
<tr>
<td>Average annual growth: 9.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### NOTES:

1. No introductions and no problem with year 2012, as not possible for any more calves to be born.
2. Therefore growth needs no adjustment whatsoever, and is accurate at +9.3%.
3. However, one bull was removed within a year of establishing the breeding group, due to continuous harassing of a cow.
4. Therefore, more accurately, he should not be included in the founder population. This results in a more realistic growth of +12.6%, moving the founder to 2003.
5. Hypothetically, if poor management helicopter actions during a census had not killed a female calf, the growth rate after adding her and any possible progeny back, would have been +13.9%.
6. For the purposes of the study, we used the end year population number for growth of +9.3% even though 12.6% would have been more realistic / accurate.
7. A total of 7 births in 10 years.
8. Serious note should be taken of the extremely poor growth for the first 4 years (as is, minus 3.75% or excluding the bull +1.25%). This was due to the fact that the remaining bull was still a sub-adult. This shows the serious consequences of small founder populations, with poor sex ratios, and a single immature bull.
Addendum to: Focus on Black Rhino: *D. b. bicornis* Population Dynamics 2002 - 2012

**Gamma Population Detailed Growth Rate Analysis:**

<table>
<thead>
<tr>
<th>(A) Year</th>
<th>(B) End Year Population Numbers</th>
<th>(C) Yearly Growth</th>
<th>(D) Adjustment for loss of one ♀ immediately after translocation &amp; release into new park.</th>
<th>(E) The loss of future offspring in 2010 &amp; 2012 due to one female’s death.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Founder:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct. 2006</td>
<td>8</td>
<td>8 7 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>7</td>
<td>-12.5%</td>
<td>8 0.0%</td>
<td>7 0.0%</td>
</tr>
<tr>
<td>2007</td>
<td>9</td>
<td>28.6%</td>
<td>10 25.0%</td>
<td>9 28.6%</td>
</tr>
<tr>
<td>2008</td>
<td>10</td>
<td>11.1%</td>
<td>11 10.0%</td>
<td>10 11.1%</td>
</tr>
<tr>
<td>2009</td>
<td>12</td>
<td>20.0%</td>
<td>13 18.2%</td>
<td>12 20.0%</td>
</tr>
<tr>
<td>2010</td>
<td>14</td>
<td>16.7%</td>
<td>15 15.4%</td>
<td>14 16.7%</td>
</tr>
<tr>
<td>2011</td>
<td>15</td>
<td>7.1%</td>
<td>16 6.7%</td>
<td>15 7.1%</td>
</tr>
<tr>
<td>May 2012</td>
<td>16</td>
<td>6.7%</td>
<td>17 6.3%</td>
<td>16 6.7%</td>
</tr>
</tbody>
</table>

**Methods of Dealing with 2012**

1. Average annual growth for 6.42 years. 12.1%

2. Worst case, ignore potential births after May 2012. 11.1%

3. Forecast of one more calf (growth 2012 = 13.3%). 12.0%

4. As a matter of interest, the death of a young female during translocation, is not only one rhino, but also her progeny. Growth could have been between 13% & 14% (see section highlighted green). This of course was ignored for the calculation of growth.

5. Although Annexure X shows 2005 with 7 and 2006 with 7 the actual number of black rhinos translocated to the new park (founder) by October 2006 was 8, but a sub-adult female died soon after release, therefore the year end number was 7.

**NOTES:**

1. The vertical column A shows populations as is, with a growth of +12.1%, taking into account the early termination. If we ignore the worst case scenario of no further calves born from May to December 2012, the growth figure is either +12.1% or 12.0%. We chose the most conservative of 12.0% for the paper.

2. However, we strongly believe that an adjustment is necessary for the one female sub-adult death immediately after translocation & release into the new park. (The old park was deproclaimed, and all the animals were relocated to the new park in September & October 2006 - a massive translocation exercise).

3. We used two methods to adjust for this one rhino, i.e. adding back to all years, or subtracting her from the founder population. The worst growth in each of these scenarios was 11.7% and 12.9%, or an average of 12.3%. If we exclude 2012, then B and C reflect growths of 12.5% and 13.9%. We selected 12.0% which is still very conservative.

4. As a matter of interest, the death of a young female during translocation, is not only one rhino, but also her progeny. Growth could have been between 13% & 14% (see section highlighted green). This of course was ignored for the calculation of growth.

5. Although Annexure X shows 2005 with 7 and 2006 with 7 the actual number of black rhinos translocated to the new park (founder) by October 2006 was 8, but a sub-adult female died soon after release, therefore the year end number was 7.
Addendum to: Focus on Black Rhino: *D. b. bicornis* Population Dynamics 2002 - 2012

**Delta Population Detailed Growth Rate Analysis:**

<table>
<thead>
<tr>
<th>Year</th>
<th>Population Numbers</th>
<th>Yearly Growth</th>
<th>Notes / Explanation of introductions &amp; removals</th>
<th>Adjustments to cope with introductions &amp; sub-adults, i.e., assume founder population to be 7 in 2006 (there were 7 rhino in 2008 after all introductions).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Founder 2005: 2</td>
<td>2</td>
<td>0.0%</td>
<td>2 sub-adult ♂</td>
<td>Founder 7</td>
</tr>
<tr>
<td>2006</td>
<td>2</td>
<td>0.0%</td>
<td>2 sub-adult ♂</td>
<td>Introduced 3 &amp; removed 1. Remaining 2 sub-adults were 1 ♂ &amp; 1 ♀.</td>
</tr>
<tr>
<td>2007</td>
<td>4</td>
<td>100.0%</td>
<td>7</td>
<td>7 0.0%</td>
</tr>
<tr>
<td>2008</td>
<td>7</td>
<td>75.0%</td>
<td>7</td>
<td>Introduced 3, all sub-adult ♀. No breeding - all sub-adults. One ♀ calf born.</td>
</tr>
<tr>
<td>2009</td>
<td>7</td>
<td>0.0%</td>
<td>7</td>
<td>7 0.0%</td>
</tr>
<tr>
<td>2010</td>
<td>8</td>
<td>14.3%</td>
<td>8</td>
<td>Two calves born. Two calves born 1 ♀ &amp; 1 ♂.</td>
</tr>
<tr>
<td>2011</td>
<td>10</td>
<td>25.0%</td>
<td>10</td>
<td>10 25.0%</td>
</tr>
<tr>
<td>2012</td>
<td>12</td>
<td>20.0%</td>
<td>12</td>
<td>12 20.0%</td>
</tr>
<tr>
<td>Average annual growth: 33.4%</td>
<td></td>
<td></td>
<td></td>
<td>No breeding for 5 years from 2005 to 2009. Then 5 calves in 3 years as population matured sexually. This figure is totally distorted due to the tiny founder (2 ♂), &amp; frequent small introductions.</td>
</tr>
<tr>
<td>Average growth: 9.9%</td>
<td></td>
<td></td>
<td></td>
<td>Note: By adjusting the founder number &amp; year, this indicates zero growth for 3 years. This is an unfair reflection of performance, as no growth was possible with a population of sub-adults. Therefore, a fairer representation of growth is over the last 3 years when rhinos reached maturity. This growth is +19.8% and is on a par with the best population in the Alpha area.</td>
</tr>
</tbody>
</table>

**Notes for all four project areas:**

To explain high growths in small populations, the often used argument is that one birth in a population of two is 50%, and so the figures are exaggerated out of all proportion. While true, it is our contention, particularly with the four populations in our project, that negative factors far outweigh the positive distortions in small populations.

**Alpha:** Very small founder and huge losses (6), as a result of one introduction. This figure is totally distorted due to the tiny founder (2 ♂), & frequent small introductions. Therefore, if planned better at the outset, these four populations would have grown even faster than the impressive record we have documented.
Addendum to : Focus on Black Rhino : \textit{D.b.bicornis} Population Dynamics 2002 - 2012

\textbf{Alpha} - Population Performance : 1999 - 2012 Actual rhinos

Annexure X

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Updated : 21 June 2013

\textbf{Black Rhinoceros} : \textit{Diceros bicornis bicornis} Actual:

\textbf{To May}

\begin{tabular}{llcccccccccccccccc}
\hline
\hline
Total & & & & 5 & 7 & 7 & 9 & 9 & 12 & 12 & 15 & 13 & 16 & 18 & 22 & 26 & 32 & 34 \\
Removed & & - 3 (2 male) & & & & & & & & & & & & & & & & \\
\hline
\end{tabular}
Addendum to : Focus on Black Rhino : *D. b. bicornis* Population Dynamics 2002 - 2012

Beta : Population Performance : 2002 - 2012 Actual rhinos

Annexure X

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Updated : 16 May 2013

Black Rhinoceros : *Diceros bicornis bicornis*

<table>
<thead>
<tr>
<th>Actual:</th>
<th>To May</th>
<th>Forecast</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Introduced</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Removed</td>
<td>0</td>
<td>-1</td>
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<tr>
<td>Deaths</td>
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<td>0</td>
</tr>
<tr>
<td>Births</td>
<td>0</td>
<td>0</td>
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</table>

Total number of rhino:

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<td>7</td>
<td>^ Introduced</td>
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</tbody>
</table>

Updated: 16 May 2013
Addendum to: Focus on Black Rhino: *D. b. bicornis* Population Dynamics 2002 - 2012

**Gamma: Population Performance: 2002 - 2012 Actual rhinos**

Updated: 16 May 2013

Gamman : Population Performance : 2002 - 2012 Actual rhinos

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### Black Rhinoceros: *Diceros bicornis bicornis*

<table>
<thead>
<tr>
<th>Actual</th>
<th>Forecast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Vaalbos</td>
</tr>
<tr>
<td>Introduced</td>
<td>8</td>
</tr>
<tr>
<td>Removed</td>
<td>1</td>
</tr>
<tr>
<td>Deaths</td>
<td>1</td>
</tr>
</tbody>
</table>

Total number of rhino:

1. Ubhejane
2. Nkombe
3. Rathie
4. Wildeman
5. Uk.Dec’00
7. Gustav
8. Tiffiny
9. Leanne
10. Bwana
11. Jabula
12. Tshukudu
13. Petrus
14. Dju-ba
15. Makoleng
16. Male
17. Female
18. (Removed)
19. *Introduced*
20. Died
21. Citooro
22. Ubhej 12/12
23. Mosu

*Updated: 16 May 2013*
### Addendum to: Focus on Black Rhino: *D.b.bicornis* Population Dynamics 2002 - 2012

**Annexure X**

**Delta: Population Performance: 2005 - 2012 Actual rhinos**

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**Black Rhinoceros: *Diceros bicornis bicornis***

**Updated: 16 May 2013**

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</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>7</td>
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<td>8</td>
<td>10</td>
<td>12</td>
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<tr>
<td><strong>Introduced</strong></td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>3</td>
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<td>0</td>
<td>0</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td><strong>Deaths</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td><strong>Births</strong></td>
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<td>2</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Total number of rhino:**

1. Kaba
2. Kaba
3. Kaba
4. Kaba
5. Kaba
6. Kaba
7. Kaba
8. Kaba
9. Kaba
10. Kaba
11. Kaba
12. Kaba

(Removed)

1. Nantie
2. Nantie
3. Nantie
4. Nantie
5. Nantie
6. Nantie
7. Nantie
8. Nantie
9. Nantie
10. Nantie
11. Nantie
12. Nantie

^ Introduced

1. Vuka
2. Dhora
3. Dhora
4. Dhora
5. Dhora
6. Dhora
7. Dhora
8. Dhora
9. Dhora
10. Dhora
11. Dhora
12. Dhora

Died

1. Thandi
2. Thandi
3. Thandi
4. Thandi
5. Thandi
6. Thandi
7. Thandi
8. Thandi
9. Thandi
10. Thandi
11. Thandi
12. Thandi

*^ Introduced:

1. Vuka
2. Dhora
3. Dhora
4. Dhora
5. Dhora
6. Dhora
7. Dhora
8. Dhora
9. Dhora
10. Dhora
11. Dhora
12. Dhora

*^ Introduced:

1. Vuka
2. Dhora
3. Dhora
4. Dhora
5. Dhora
6. Dhora
7. Dhora
8. Dhora
9. Dhora
10. Dhora
11. Dhora
12. Dhora

*^ Introduced:
Demography of Wild Asian elephants in Southern Sri Lanka Estimated Through Individual-based Longitudinal Monitoring

Shermin de Silva
Postdoctoral Fellow - Colorado State University (Wittemyer lab)

International Elephant & Rhino Conservation Symposium 2013
Study Site

Uda Walawe National Park
Sri Lanka
~308 sq. km

Map: Fernando et al., Gajah, 35 2012
Habitats
We want to assess...

- Population size / density
- Age structure
- Age at first reproduction
- Sex ratios
- Inter-birth intervals
- Fecundity
- Mortality by age class and sex

WHY? - Understand/model population dynamics
- Ecosystem health
Data Collection
Age/size classes - Young

de Silva et al. 2011, Biol. Cons.
## Age/size classes – Adult Female

<table>
<thead>
<tr>
<th>Age class</th>
<th>Gross attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-15</td>
<td>Rounded head shape and ears, square body in profile, rounded pelvic region/base of tail (rump). Primiparous females begin to show breast development during pregnancy.</td>
</tr>
<tr>
<td>16-30</td>
<td>Lengthening of back and ears to assume more rectangular shape, head retains similar size in proportion to body as in sub-adults. Squaring of pelvic/base of tail region, protrusion of spinal, pelvic and basal tail bones, (generally) well-developed breasts. One or more dependent calves evident.</td>
</tr>
<tr>
<td>31-50</td>
<td>Hollowing of cheek cavity and forehead above brow and temples, enlargement of head in proportion to body size particularly of domes on upper cranium (not always evident), splaying of toes.</td>
</tr>
<tr>
<td>&lt;50</td>
<td>Loss of body mass especially around skull and spine, protrusion of cheek bones and temple, hollow cheeks when mouth is closed, (i.e. when not chewing, indicative of lost teeth). May begin to show reduction in breast size unrelated to loss of a calf.</td>
</tr>
</tbody>
</table>

Oldest age class 60+ yrs!

de Silva et al. 2013, *PLOS One*, in revision
The basics...

de Silva et al. 2011, Biol. Cons.
Spatially-explicit density estimation, SPACECAP for R

Current IDs (Adults + subs):

~ 409 Males
~ 465 Females

Estimated superpopulation: 800-1160

Estimated superpopulation: >3.5/sq. km

de Silva et al. 2011, Biol. Cons.
Birth seasonality

de Silva et al. 2013, PLOS One, in revision
Primiparity

Deepika, Dinusha & Deepika’s baby
2005

Deepika, Dinusha & Deepika’s calf
2009
Primiparity

Deepika, Dinusha & Deepika’s baby 2005

Est. age at first conception: **11.5 yrs** (N=21)

Dinusha, her first baby Enzo & sub-adult female (Dinusha’s sister), 2011
## Inter-birth intervals

<table>
<thead>
<tr>
<th>Sample criterion</th>
<th>Time Interval</th>
<th>N females</th>
<th>N births</th>
<th>Avg. IBI (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Births after 2005 recorded within 2 months</td>
<td>2006-2012 (~6.5 yrs)</td>
<td>13</td>
<td>26</td>
<td>47.5 ± 11.6</td>
</tr>
<tr>
<td>All births after 2005</td>
<td>2006-2012 (7 yrs)</td>
<td>27</td>
<td>54</td>
<td>47.2 ± 10.3</td>
</tr>
<tr>
<td>Births after 2005, mortalities within 1 yr excluded</td>
<td>2006-2012 (7 yrs)</td>
<td>22</td>
<td>44</td>
<td>50.7 ± 7.5</td>
</tr>
<tr>
<td>Females with calves &lt; 3 yrs old in 2005</td>
<td>2003-2012 (10 yrs)</td>
<td>37</td>
<td>78</td>
<td>53.3 ± 14.5</td>
</tr>
</tbody>
</table>

*de Silva et al. 2013, PLOS One, in revision*
# Inter-birth intervals

<table>
<thead>
<tr>
<th>Sample criterion</th>
<th>Time Interval</th>
<th>N females</th>
<th>N births</th>
<th>Avg. IBI (months)</th>
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<tbody>
<tr>
<td>Births after 2005 recorded within 2 months</td>
<td>2006-2012 (~6.5 yrs)</td>
<td>13</td>
<td>26</td>
<td>47.5 ± 11.6</td>
</tr>
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<td>2006-2012 (7 yrs)</td>
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</tr>
</tbody>
</table>

IBI of five females who lost newborns within 1 yr ~ 19 months
Fecundity

Female offspring per female per year

Age class

Fecundity, \( m(x) \)

0.3
0.25
0.2
0.15
0.1
0.05
0
1-10 11-20 21-30 31-40 41-50 51-60 60<

de Silva et al. 2013, *PLOS One*, in revision
## Sex ratios

<table>
<thead>
<tr>
<th>Age class of mother</th>
<th>Females</th>
<th>Males</th>
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<tbody>
<tr>
<td>11-15</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>16-20</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>21-30</td>
<td>5</td>
<td>8</td>
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<td>31-40</td>
<td>14</td>
<td>4</td>
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<tr>
<td>41-50</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>50&lt;</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>32</td>
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### At birth

<table>
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<tr>
<th>Age class</th>
<th>Female</th>
<th>Male</th>
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<tr>
<td>0-2</td>
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<td>13</td>
</tr>
<tr>
<td>3-5</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>6-10</td>
<td>57</td>
<td>37</td>
</tr>
<tr>
<td>11-15</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>16-20</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>21-30</td>
<td>31</td>
<td>-</td>
</tr>
<tr>
<td>31-40</td>
<td>34</td>
<td>-</td>
</tr>
<tr>
<td>41-50</td>
<td>28</td>
<td>-</td>
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<tr>
<td>51-60</td>
<td>21</td>
<td>-</td>
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<tr>
<td>61-60</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>274</td>
<td>99</td>
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</table>

### Herd composition

<table>
<thead>
<tr>
<th>Age class</th>
<th>Female (%)</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>(4.7)</td>
<td>13</td>
</tr>
<tr>
<td>3-5</td>
<td>(11.3)</td>
<td>29</td>
</tr>
<tr>
<td>6-10</td>
<td>(20.8)</td>
<td>37</td>
</tr>
<tr>
<td>11-15</td>
<td>(9.5)</td>
<td>20</td>
</tr>
<tr>
<td>16-20</td>
<td>(6.2)</td>
<td>-</td>
</tr>
<tr>
<td>21-30</td>
<td>(11.3)</td>
<td>-</td>
</tr>
<tr>
<td>31-40</td>
<td>(12.4)</td>
<td>-</td>
</tr>
<tr>
<td>41-50</td>
<td>(10.2)</td>
<td>-</td>
</tr>
<tr>
<td>51-60</td>
<td>(7.7)</td>
<td>-</td>
</tr>
<tr>
<td>&gt;60</td>
<td>(5.8)</td>
<td>-</td>
</tr>
</tbody>
</table>

### Est. adult sex ratio

F : M ~ 1.18

---

de Silva et al. 2013, *PLOS One*, in revision
Injuries & Mortalities

Attempted capture

Snares

de Silva et al. 2013, *PLOS One*, *in revision*
Injuries & Mortalities

Burns

de Silva et al. 2013, *PLOS One, in revision*
Injuries & Mortalities

Gunshots

de Silva et al. 2013, *PLOS One*, in revision
Injuries & Mortalities

Poison?

Accidents

Starvation?

del Silva et al. 2013, *PLOS One, in revision*
Males significantly (3x) more likely to exhibit injury or death through human activity.

dev Silva et al. 2013, *PLOS One*, in revision
Injuries & Mortalities

de Silva et al. 2013, *PLOS One*, in revision
1. Tracking growth & dispersal
2. MORTALITY RATES???
3. Males
4. Long-term dynamics
Acknowledgements

Colleagues & advisors:
Dr. Devaka Weerakoon – University of Colombo
Dr. UK Padmalal – Open University of Sri Lanka
Dr. Dorothy Cheney – University of Pennsylvania
Dr. Sergey Kryazhimskiy – Harvard University
Dr. George Wittemyer – Colorado State University
Dr. Jonathan Dushoff – McMaster University
Dr. Naomi Pierce – Harvard University
Dr. Benoit Goossens & Nurzhafarina Othman, Danau Girang Field Center.

Department of Wildlife Conservation, Sri Lanka:
Mr. SRB Disannayake, Mr. MR Mohammed
Uda Walawe National Park wardens & personnel

Field staff, students and other personnel:
US Weerathunga, TV Kumara
CE Webber, L Snyder
DN Suhood, Anula, Jayasena

International Elephant Foundation
THANK YOU!

http://elephantresearch.net (Sri Lanka)
http://trunksnleaves.org (USA)
Determining Potential Environmental and Social Factors Affecting the Success of the Black Rhinoceros in Addo Elephant National Park, South Africa

Rachel Santymire, Jordana Meyer, Evan Sorley, Jed Bird, Bruce Schulte, & Elizabeth Freeman
Background: Black Rhino Natural History

~65,000

2,000 by 1992
- Due to poaching & habitat loss

Now, ~5,000 (IUCN, 2013)
- Critically endangered
Background: Black Rhino Biology

- Herbivore: Browser (eats shrubs/trees) using prehensile lip
- Weigh ~3,000 lbs
- Horns (n=2) composed of keratin
- In same family as the horse (odd-toed; perissodactyla)
- Solitary except female with calves
- Use middens to mark territory and for communication
- Crepuscular (active evening/mornings)
What factors are influencing black rhino populations?
What factors are influencing black rhino populations?
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What factors are influencing black rhino populations?

- Rhinos shift their activity patterns in response to elephant presence (Tambling et al. In prep)

- Rhinos shift diet in areas where elephants have altered the vegetation (Landman & Kerley In Press; Landman et al. In Press)
What factors are influencing black rhino populations?

Environmental conditions
What factors are influencing black rhino populations?

Anthropogenic activity
So how are all of these factors affecting rhino behavior, physiology and ultimately population success?
Hormonal analysis provides valuable information about factors influencing free-ranging wildlife population dynamics.

• Gonadal hormones (for reproduction)
• Adrenocortical hormones (for stress physiology)
• Monitor the health status of wildlife
• Assist with management and conservation decision-making by providing information
Our goal is to establish a health monitoring program that will investigate the relationship among black rhino hormonal activity and ecological factors that vary between two sections of Addo Elephant National Park (AENP).
• In 1931 its goal was to protect the remaining 11 elephant. Now there are >400 individuals!!
• AENP will be the third largest national park in South Africa.
1995 AENP reintroduced south-western arid subspecies of black rhino (*Diceros bicornis bicornis*)

Now has ~70% of South Africa’s population

Addo Population (21)
- 9 males, 10 females and 2 unknown

Nyathi Population (24)
- 12 males, 10 females, 2 unknown
# Environmental Factors

<table>
<thead>
<tr>
<th>Factors</th>
<th>Addo</th>
<th>Nyathi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio</td>
<td>Female-biased</td>
<td>Male-biased</td>
</tr>
<tr>
<td>Elephant density</td>
<td>High (~300)</td>
<td>Moderate (~100)</td>
</tr>
<tr>
<td>Predators</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Vegetation</td>
<td>Limited</td>
<td>Abundant</td>
</tr>
<tr>
<td>Tourism</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Size of section</td>
<td>11,500 ha</td>
<td>14,000 ha</td>
</tr>
</tbody>
</table>
Field Methods: Looking for Rhinos
Looking for rhinos in AENP
Rhino Middens
Camera traps Set Up on Middens

Identify individuals by:
- ear notch
- horns

Get sample date and time
Fecal Sample Collection

Endocrine analysis

Parasite load
Modifying methods for the field

LAB

Homogenize

- All equipment is battery-powered
- Very cost effective
- Simplifies storage
- Simplifies transportation
- Simplifies treatment for disease

(Santymire & Armstrong 2010 in Zoo Biology)
Factors Analyzed

- Park Section
  - Addo
  - Nyathi
- Type of road
  - Staff
  - Concession
- Landscape cover
  - Xeric
  - Mesic
- Season
  - Wet
  - Dry
- Number of rhinos
Camera Trap Locations

- Grouped our camera traps with at least 10 samples
- We collected 167 fecal samples
Preliminary Stress Results
Park Section

Glucocorticoid metabolites (ng/g feces)

Addo

Nyathi

P < 0.001

a

b
Preliminary Stress Results
Park Section

P < 0.001
Preliminary Stress Results
Landscape cover-Addo only

Glucocorticoid metabolites (ng/g feces)

Xeric

Mesic

P >0.05
Preliminary Stress Results
Type of road - Addo only

Glucocorticoid metabolites (ng/g feces)

P > 0.05
Preliminary Stress Results

Season

Glucocorticoid metabolites (ng/g feces)

P = 0.05

Dry

Wet

a

b
### Preliminary Stress Results

**Season and number of rhinos**

<table>
<thead>
<tr>
<th># of rhinos</th>
<th>C-trap location</th>
<th>Season</th>
<th>Waterhole</th>
<th>FGM (ng/g)</th>
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<tr>
<td>7 (3.4)</td>
<td>N. Addo</td>
<td>Wet</td>
<td>Yes</td>
<td>211.1 ± 20.1</td>
</tr>
<tr>
<td>4 (2.2)</td>
<td>S. Addo</td>
<td>Dry</td>
<td>No</td>
<td>179.3 ± 27.1</td>
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<td>Dry</td>
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<tr>
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<td>Dry</td>
<td>Yes</td>
<td>70.1 ± 12.6</td>
</tr>
<tr>
<td>4 (3.1)</td>
<td>West Nyathi</td>
<td>Wet</td>
<td>Yes</td>
<td>395.1 ± 68.0</td>
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</tbody>
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</tbody>
</table>
Summary

• Rhinos in Addo had higher FGMs than Nyathi rhinos.

• In Addo, landscape cover and type of roads did not influence FGM values; however, couldn’t test tourist roads.

• FGMs were higher in wet versus dry season, but season did not seem to influence the number of rhinos at each site even the two sites with waterholes.

• FGM values were not influenced by the number of different individual visitors nor by sex.
Conclusions

• We are limited by the c-traps on middens.
• Rhinos may be avoiding the highly disturbed areas
• Addo rhinos did have higher FGMs than Nyathi
  • Inter-species conflicts (elephants and lions)
  • Calving interval longer in Addo by ~1 year
  • Addo rhinos have fewer commensal parasites
• Results can be used to assist AENP managers with improving the success of their rhino populations.
Next Steps

- Extract DNA from feces to identify “unknown” samples and possibility determine paternity
Acknowledgments

- Co-authors (Jordana Meyer, Evan Sorley, Bruce Schulte and Elizabeth Freeman)
- John Addendorf, SANparks AENP Conservation Biologist
- Angela Gaylord, SANparks Regional Ecologist
- Thando Mendela, SANparks Research Assistant
- Maggie Hook, WKU MS student
- Maggie Wisniewska, WKU MS student
- Guides at River Bend Lodge, Gorah Lodge, Nguni Lodge and AENP
- Diana Armstrong, LPZ
White rhinoceros reproduction: Insights from the wild and semi-wild

Ron Swaisgood, Lisa Nordstrom, Shannon Chapman
San Diego Zoo Global Institute for Conservation Research

Thanks to:
Amerman Fund
IRF
SOS Rhino
Dr. Helen Raney
Heller Foundation
Driving question behind research: Why do captive-born female white rhinos fail to reproduce?
Testing hypotheses for Reproductive Failure of Captive-born Females

Reproductive Suppression
  By the mother
  By other (older, dominant) wild-caught females
Enclosure Size
Social Group Composition
Nutrition

Methods

Daily observations of SDWAP rhinos
3 hours/day, 7 days/week, 4 years
5 captive-born, 6 wild-caught

40 captive-born
28 wild-caught

Location effect constant

Any deficiencies in captive-born females?

- No. About 30 graphs show normal behavioral estrus, aggression, courtship and copulation.
- No differences between wild-born and captive-born.

Any effects of other females on captive-born female reproduction?

- No evidence for female-female dominance
- Presence of mother or other (older) wild-caught females increases reproduction

$F_1 = 34\%$

$F_0 = 78\%$

$p = 0.003$

Control for

--location,

--years of opportunity

--copulation

Conclusions

- Reproductive suppression is not the cause of the F1 problem.
- Weak link is postcopulatory
- It’s the development

Weak Links in the Chain of Events Leading to Successful Reproduction

Behavioral Estrus → Endocrine Cycling → Male Courtship → Female Receptivity → Copulation → Ovulation → Conception → Pregnancy

OK OK OK OK

?
Reproductive cycles

- Behavioral observations
- Radioimmunoassay for progesterone metabolites in feces

## Individual cycle lengths (days)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Type I Cycles</th>
<th>Type II Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dumisha</td>
<td>Interluteal</td>
<td>Interluteal</td>
</tr>
<tr>
<td></td>
<td>Luteal</td>
<td>Luteal</td>
</tr>
<tr>
<td></td>
<td>Cycle</td>
<td>Cycle</td>
</tr>
<tr>
<td>Dumisha</td>
<td>16</td>
<td>8.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dumisha</td>
<td>8.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56</td>
</tr>
<tr>
<td>Michelin</td>
<td>8</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>44.5</td>
</tr>
<tr>
<td>Michelin</td>
<td>8.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>50.5</td>
</tr>
<tr>
<td>Mjuba</td>
<td>8.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>32.5</td>
<td>66.5</td>
</tr>
<tr>
<td>Sinyaa</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>61</td>
</tr>
<tr>
<td>Sinyaa</td>
<td>4</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>60.5</td>
</tr>
<tr>
<td>Ujima</td>
<td>18.5</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>19.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.1</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>65.6</td>
</tr>
<tr>
<td>Ujima</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Ujima</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23.5</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>9.3</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>25.7</td>
<td>55.1</td>
</tr>
<tr>
<td></td>
<td>35.0</td>
<td>65.6</td>
</tr>
<tr>
<td>SEM</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>2.1</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>2.1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Ultrasound findings:

- These long cycles were not always cycles...sometimes they were pregnancy and early embryonic death.
- They also had chronic uterine infections.
Normality must be defined:
• In the field
• In a reproductively healthy population
iMfololzi Game Reserve
South Africa
Radiotracked 16 female rhinos for 2+ years
Each female tracked 3X/week
Reproductive cycles

- Documented 17 cycles in 6 females
- All were approximately 30 days (25-36)
- Short cycles are normal
Female ranging patterns: implications for mating strategies

Female home range overlap with male territories

- “Passive” female mate choice
- Mate with familiar males

Where does reproductive breakdown occur on the C-W continuum?

Captive  Semi-wild  Wild
South Africa game reserve survey

- Interviewed managers at 96 “game farms”
- 7-page questionnaire
- Data for 234 individual females on 47 properties
- Largest detailed database on rhinos on private reserves
Characteristics of game reserves

- Fenced, managed
- Natural and exotic plant communities
- Varying space
  - 1 to 1250 ha
- Variable social density
  - 0.0006/ha to 2.0/ha
- Variable pop size
  - 2-111; mean = 12
- “Age” of rhino reserve
  - 1-40; mean = 12
- Calves born on property
  - 0-130; mean = 8.3
Is there a difference in reproduction between $F_0$ and $F_1$ females in semi-wild white rhinos?

No differences

<table>
<thead>
<tr>
<th></th>
<th>$F_0$</th>
<th>$F_1$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repro rate</td>
<td>$0.30 \pm 0.17$</td>
<td>$0.30 \pm 0.14$</td>
<td>0.94</td>
</tr>
<tr>
<td>IBI</td>
<td>32.3</td>
<td>30.7</td>
<td>0.94</td>
</tr>
<tr>
<td>% Repro</td>
<td>92%</td>
<td>98%</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>113</td>
<td>117</td>
<td></td>
</tr>
</tbody>
</table>

cf. F1 repro rate in NA zoos = 0.01 ca. 2008

95% of properties have F1 reproduction
What factors in the adult environment influence reproduction in semi-wild white rhinos?

<table>
<thead>
<tr>
<th>Environmental Factor</th>
<th>Statistic</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern of Supplemental Feeding</td>
<td>$F_{3,79}=0.61$</td>
<td>$p=0.611$</td>
</tr>
<tr>
<td>Supplemental Feeding Intensity</td>
<td>$F_{3,75}=1.58$</td>
<td>$p=0.201$</td>
</tr>
<tr>
<td>Supplemental Feed Combined Scale</td>
<td>$F_{2,71}=6.60$</td>
<td>$p=0.002$</td>
</tr>
<tr>
<td>Male Social Environment</td>
<td>$F_{1,76}=0.00$</td>
<td>$p=0.949$</td>
</tr>
<tr>
<td>Female Social Environment</td>
<td>$F_{1,76}=0.00$</td>
<td>$p=0.999$</td>
</tr>
<tr>
<td>Overall Social Density</td>
<td>$F_{1,68}=5.26$</td>
<td>$p=0.025$</td>
</tr>
<tr>
<td>Male Social Density</td>
<td>$F_{1,81}=0.00$</td>
<td>$p=0.993$</td>
</tr>
<tr>
<td>Female Social Density</td>
<td>$F_{1,68}=5.25$</td>
<td>$p=0.025$</td>
</tr>
<tr>
<td>Rainfall</td>
<td>$F_{1,80}=0.84$</td>
<td>$p=0.361$</td>
</tr>
<tr>
<td>Property Size</td>
<td>$F_{1,81}=0.52$</td>
<td>$p=0.474$</td>
</tr>
</tbody>
</table>

**Analysis of $F_1$ and $F_0$ females combined**
Relationship between social density and reproductive rate

Density of all rhinos

Density of adult female rhinos

Fig. 1a—Social density during adulthood and reproductive rate

Fig. 1b—Adult female social density during adulthood and reproductive rate
Supplemental feeding increases reproductive rate

**Fig. 2** - The effect of level of supplemental feed (a combined measure of feed pattern and intensity) on reproductive rate as mean reproductive rate ± standard error.
What environmental factors during development influence reproduction in semi-wild white rhinos?

<table>
<thead>
<tr>
<th>Environmental Factor</th>
<th>Statistic</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplemental Feeding Pattern&lt;sup&gt;a&lt;/sup&gt;</td>
<td>F&lt;sub&gt;1,23&lt;/sub&gt;=3.590</td>
<td>p=.071</td>
</tr>
<tr>
<td>Supplemental Feeding Intensity&lt;sup&gt;b&lt;/sup&gt;</td>
<td>F&lt;sub&gt;1,22&lt;/sub&gt;=.304</td>
<td>p=.587</td>
</tr>
<tr>
<td>Male Social Environment</td>
<td>F&lt;sub&gt;1,16&lt;/sub&gt;=.085</td>
<td>p=.774</td>
</tr>
<tr>
<td>Female Social Environment</td>
<td>F&lt;sub&gt;1,17&lt;/sub&gt;=.250</td>
<td>p=.624</td>
</tr>
<tr>
<td>Overall Social Density</td>
<td>F&lt;sub&gt;1,8&lt;/sub&gt;=19.354</td>
<td>p=.003</td>
</tr>
<tr>
<td>Male Social Density</td>
<td>F&lt;sub&gt;1,15&lt;/sub&gt;=9.754</td>
<td>p=.007</td>
</tr>
<tr>
<td>Female Social Density</td>
<td>F&lt;sub&gt;1,8&lt;/sub&gt;=22.604</td>
<td>p=.002</td>
</tr>
<tr>
<td>Rainfall</td>
<td>F&lt;sub&gt;1,18&lt;/sub&gt;=.078</td>
<td>p=.783</td>
</tr>
<tr>
<td>Property Size</td>
<td>F&lt;sub&gt;1,21&lt;/sub&gt;=1.649</td>
<td>p=.214</td>
</tr>
</tbody>
</table>
Developmental social density effects on F1 reproduction

Fig. 3a - Social density during development and reproductive rate.

Fig. 3b - Adult male social density during development and future reproductive rate.

Fig. 3c - Adult female social density during development and reproductive rate.
Tentative conclusions

- Increased social density is good for adult females
- Bad for developing females
- Social environment has lasting developmental effects

Why?
  - Nutritional limitation/competition
  - Social stress
Other findings

- Single-male, multi-female reserves had normal reproduction
- 1.1 (Noah’s ark) did not reproduce, even with large space in native habitat
- Mean AFB = 7.5y
- Mean AF Cop = 6.0y
- Mean AF Court = 5.2y
The view from Africa...

- Normal cycle is 30 days
- No F1 problem in small game reserves
- No female reproductive suppression
- Conspecific density influences reproduction
  - In complicated ways
- Nutrition influences reproduction
The way forward...

- Remaining plausible hypotheses:
  - Diet during development
    - Phytoestrogens (Tubbs et al. 2012)
  - Social interactions during development
    - Precocious copulation

Hey! It’s the hay!
More answers on the horizon...
Attempt to control estrus and ovulation in white rhinoceroses using a synthetic progestagen and slow-release GnRH analogue.

Annemieke van der Goot, University of Western Australia
THE WHITE RHINO BREEDING PROBLEM

In the wild successful breeding in many areas

In captivity not enough pregnancies
- Low fertility rate
- <50% of ♀♀ breeds
- F1 less then F0
- Captive population not self sustaining
REPRODUCTION IN CAPTIVITY

- For once we can’t blame the male
- The captive white rhino population is not growing ($\lambda = 1.001$)
- Only 18-39% of captive-born (F1) females reproduce
- A problem observed globally in many (semi-)captive settings
- Causative factors and underlying mechanism yet unclear
- Observed irregular cycling patterns (variation in cycle duration)
- High incidence of anovulation, acyclicity and pathologies

ONE APPROACH is to IMPROVE OVULATION RATE

HERMES ET AL 2012; FOOSE & WIESE 2006; SWAISGOOD ET AL 2006; HERMES ET AL 2006
UNDERSTANDING THE ESTROUS CYCLE

- Illustration of estrous cycle of a mare (Perissodactilae)
ADVANCED BREEDING TECHNOLOGIES

General need amongst white rhino breeding facilities

- Improving pregnancy rate
- More efficient timed management
- AI & greater effective use bulls

Estrus induction in white rhinoceroses

- Attempts since 1995 - success rate 0-30%
- Recent report: Chlormadinone acetate with hCG/GnRH analogue
  - Anovulatory and ovulatory females in Europe
  - Higher success rate

HERMES ET AL 2012; HERMES ET AL 2006; WALZER ET AL 1995
OUR GOAL

- To provide a tool towards enhanced breeding success in the white rhinoceros
- By developing a functional and practical ovulation induction protocol that can be generally used for timed breeding management in zoos and breeding facilities worldwide

AIM OF THIS STUDY

- To induce estrus and ovulation in a female white rhinoceros by using Regu-mate (a synthetic progestagen) and Sucromate Equine (a 48h-release GnRH analogue)
### STUDY ANIMALS

<table>
<thead>
<tr>
<th>Female 1</th>
<th>1994</th>
<th>F1</th>
<th>Jacksonville Zoo</th>
<th>Multiparous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female 2</td>
<td>1992</td>
<td>F1</td>
<td>Birmingham Zoo</td>
<td>Multiparous</td>
</tr>
<tr>
<td>Female 3</td>
<td>2000</td>
<td>F2</td>
<td>Birmingham Zoo</td>
<td>Nulliparous</td>
</tr>
</tbody>
</table>

**Setting Jacksonville Zoo**

1 male + 1 female

**Setting Birmingham Zoo**

2 females + new male introduction
## OUR PROTOCOL

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>Dose /frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>-90 to 1</td>
<td>Fecal sample collection* &amp; behavioral observation</td>
<td>3 times weekly</td>
</tr>
<tr>
<td>1-21</td>
<td>Oral progesterone (Regu-mate)</td>
<td>0.022 mg/kg once daily (n=2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.044 mg/kg once daily (n=1)</td>
</tr>
<tr>
<td>30.5</td>
<td>GnRH analog 48h-release (Sucromate Equine)</td>
<td>2.5 μg/kg single IM injection</td>
</tr>
<tr>
<td>1 to 51</td>
<td>Fecal sample collection &amp; behavioral observation</td>
<td>daily</td>
</tr>
<tr>
<td>21 to 36</td>
<td>Rectal ultrasound examination</td>
<td>n=1 (Jacksonville Zoo)</td>
</tr>
</tbody>
</table>

*EMZYME IMMUNOASSAY WITH ANTIBODY RAISED AGAINST 11 ALPHA-HEMISUCCINATE CROSS-REACTING WITH P4 AND 5 ALPHA-PREGNANAN
GABBY’S ULTRASOUND
JACKSONVILLE ZOO

Mandi Schook – ultrasound specialist
**FECAL PROGESTAGENS**

*What we found*

![Graph showing changes in progesterone metabolites over time](image)

- **Post-GNRH Injection**
  - Black dots represent progesterone metabolite levels before and after a GnRH injection.
  - The graph shows a significant increase in metabolite levels following the injection.

- **Regumate**
  - Blue dots indicate the effect of Regumate on progesterone levels.
  - The graph shows a moderate increase in metabolite levels with Regumate.

- **None**
  - Red dots represent the baseline levels without any treatment.

The graph clearly illustrates the impact of GnRH injection and Regumate on progesterone metabolite levels in feces.
FECAL PROGESTAGENS

What we found

Post-GnRH
GnRH - injection
Progesterone metabolite (ng/g)

Date

6/05/13 20/05/13 3/06/13 17/06/13 1/07/13 15/07/13 29/07/13

Progestosterone metabolite (ng/g)

None
Regumate
Post-GnRH

GnRH - injection
CONCLUSION & DISCUSSION

- No mating / estrus behavior detected
- Fecal P did show synchronized luteal activity
- To improve this study we need to:
  1. Replicate samples (2-3 x sample) to avoid test errors
  2. Replicate Regu-mate treatment in one individual (without GnRH)
  3. A longer “post-GnRH” fecal sample collection period
  4. Frequent ultrasound
  5. More individuals
  6. Fine-tune doses used and timing
South-East Zoo Alliance for Reproduction & Conservation (SEZARC)
~
Jacksonville Zoo
Birmingham Zoo
Gabby, Laptop & Ajabu
~
University of Western Australia
~
Institute for Breeding Rare & Endangered African Mammals
PRESENTATIONS
SESSION V
VETERINARY CARE
MANUAL RESTRAINT AND CHEMICAL IMMOBILIZATION WITH XYLAZINE/KETAMINE OF WILD AND CAPTIVE SUMATRAN ELEPHANTS (Elephas maximus sumatranus) UNDER FIELD CONDITIONS

Christopher Stremme, Anhar Lubis, Muhammad Wahyu
Veterinary Society for Sumatran Wildlife Conservation
Hobbles / Ropes
NECK RESTRAINT
TRADITIONAL "KA"
NECK RESTRAINT / ROPES AND CHAINS
USING TREE TRUNKS AS TETHER POINTS FOR MANUAL RESTRAINT WITH NECK AND FOOT FIXATION
USING TREE TRUNKS AS TETHER POINTS FOR MANUAL RESTRAINT WITH NECK AND FOOT FIXATION
USING TREE TRUNKS AS TETHER POINTS FOR MANUAL RESTRAINT WITH NECK AND FOOT FIXATION
Manual restraint / Kunki elephants
ONE SIDED CRADLE / RUNK
DOUBLE SIDED CRADLE / DEAD END
DOUBLE SIDED CRADLE / OPEN END
CHEMICAL IMMOBILIZATION

The only tranquilizers legally and reliably available on the market in Indonesia are XYLAZINE and KETAMINE. These drugs have been proven to be sufficient for reliable standing sedation in captive and wild Sumatran elephants for different needs.
CAPTIVE ELEPHANTS
LIGHT STANDING SEDATION MAINLY IN UNRELIABLE TRAINED ELEPHANTS E.G. FOR:

• Transportation,
• Trimming tusk
• Treatments and examinations
CAPTIVE ELEPHANTS

DEEP STANDING SEDATION IN COMBINATION WITH LOCAL ANESTHETICS FOR BASIC SIMPLE SURGICAL PROCEDURES SUCH AS:

- Drainage of large abscess
- Tail amputations
- Removal of tumors
CAPTIVE ELEPHANTS

Dosages of: **0,08 – 0,15mg Xylazine/kg BW** combined with: **0,03 – 0,06 mg Ketamine/kg BW** i.m. or i.v. are used.

If prolonged sedation is needed in cases of time consuming treatments and surgery, a second injection with 1/3 to 1/2 of the initial dose can be administered about 60 to 90 min after the first injection.

In cases of light sedation (i.e. for transporting untrained elephants) about 2-4 hours after the first injection.
WILD ELEPHANTS
DEEP STANDING SEDATION FOR:
Treatment of Injuries in Wild Elephants
WILD ELEPHANTS

DEEP STANDING SEDATION FOR:

Fitting GPS Collars:
WILD ELEPHANTS

STANDING SEDATION FOR: Capture for translocation
WILD ELEPHANTS

Initial dosages of:

**0,16 – 0,36 mg Xylazine/kg BW** combined with:

**0,08 – 0,14 mg Ketamine/kg BW**

are used, administered i.m. by dart gun, blow pipe or manual.

In some cases 30 to 45 min after the initial injection, a second injection with dosages of:

**0,06 – 0,2 mg Xylazine/kg BW** and **0,02 – 0,07 mg Ketamine/kg BW**

have been administered to achieve adequate tranquilization.
REVERSAL

A dosage of: 0,05 – 0,11mg Yohimbine / kg BW i.v. is sometimes used as reversal about 45 to 75 minutes after the administration of the tranquilizer.
I thank Elephant Family, Benindi Fund, AES and the US Fish and Wildlife Service their funding support helping to enable Vesswic’s veterinary works.

I thank the national and provincial Nature conservation Agencies (PHKA and BKSDA), the camp managements and mahouts for the good collaboration.

I thank the Pittsburgh Zoo & PPG Aquarium for inviting me to this symposium and presenting some of our works in Sumatra.
OK GUYS, THANKS!

AND LET'S THANK THESE PEOPLE FOR THEIR ATTENTION OK...
URINARY HORMONE CONCENTRATIONS AND PHARMACOKINETICS/PHARMACODYNAMICS OF HALOPERIDOL IN A FEMALE INDIAN RHINOCEROS (*Rhinoceros unicornis*)

ANRI BENCO¹,², MARK CAMPBELL¹, MAJORIE BARTHEL¹, CARLOS PINTO², KATHERINE MACKINNON¹ & MONICA STOOPS¹

¹CENTER FOR CONSERVATION AND RESEARCH OF ENDANGERED WILDLIFE, CINCINNATI ZOO & BOTANICAL GARDEN

²COLLEGE OF VETERINARY MEDICINE, OHIO STATE UNIVERSITY
Female Indian Rhino “Manjula”

- DOB 10/25/2005
- Urinary hormone and ultrasound analysis from 12/2009-2/2012 indicated that female should be exhibiting regular estrous cycles
  - 7 follicular phases were observed
  - However, no regular cycles or ovulations were recorded
- Demonstrated periods of acyclicity during the spring and summer of 2010 and 2011
Female Indian Rhino “Manjula”

- Lack of normal estrous cycles due to
  - Attainment of puberty
  - Difficulty in acclimating to new surroundings
- Female Indian rhinoceros reach sexual maturity between 4-6 years
- Youngest age at conception in captivity: 2 years and 4 months
Cortisol and Puberty

- Brahman-crossbred heifers (excitable temperament compared to other breeds)
  - Reach puberty later
  - Stimulated secretion and circulating concentrations of ACTH and cortisol impair mechanisms responsible for puberty establishment
Effects of Cortisol on Reproduction

- Study by Breen et al. (2005):
- Cortisol infusions in sheep simulating one-third, one-half and maximal plasma cortisol concentrations that would be induced by isolation stress
- Infusions during early and mid-follicular phases
Effects of Cortisol on Reproduction

- **Results:**
  - Suppression of LH pulse frequency
  - Delays or prevents estradiol peak
  - Delays or blocks LH and FSH surges

- **Use of LAN’s in non-domestic species during assisted reproduction** have resulted in easier handling and significantly lower cortisol levels just before oocyte collection
Haloperidol

- Antipsychotic and tranquilizing agent
- Long-acting neuroleptic: 10-12 hour duration
- Can be orally administered
- Short and long-term use in wildlife
  - Bongo Antelope – 1 mg/kg PO SID
  - Mongolian Wild Horse – 0.3 mg/kg PO SID
  - Elephants – 40-100 mg PO BID
  - Recommended for GOHR – 0.05- 0.1 mg/kg PO with max 16 hr duration
Haloperidol

- Does not cause hypothermia or hypotension
  - Side effects: extra-pyramidal side effects have been seen (especially when further stressed with hyperthermia, noise and excitement during transportation) – rare and transient

- Studies have shown that haloperidol administration is associated with an increase in prolactin secretion - however, we did not anticipate this would negatively impact Indian rhino estrous cycles
Pharmacokinetics

- **Bongo Antelope**
  - Peak behavioral effects 2 hr post dose, peak serum 10 hr post dose
  - Haloperidol absorbed gradually and reliably from the GI tract even in the presence of food

- **Sprague-Dawley rats**
  - Significant amount of haloperidol radioactivity in urine within 8 hours of administration
  - Clear GI tract by 72 hours

- **Humans**
  - Mean elimination t1/2: 17.9 ± 6.4 hr
  - Time lag before absorption: 0.82 ± 0.25 hr
  - Bioavailability: 0.65 ± 0.14
  - Extensive tissue distribution
Objectives

- Use Haloperidol to alleviate the negative physiological effects of temperament on:
  - Reproduction
  - Exhibit behavior

- Compare urinary cortisol concentrations
  - Urine was collected in morning
  - Diurnal variations
Objectives

- Compare behavioral correlates related to public exhibition and handling for reproductive assessment (ultrasonography)
- Haloperidol assay and validation
  - Commercially available enzyme-linked immunoassay (Neogen, Lexington, KY)
- Haloperidol pharmacokinetics and pharmacodynamics
Haloperidol Dosing

- Oral doses of multiple 10 mg tablets
  - Concealed in a banana and hand-fed to rhino every morning
  - Effect during peak exhibit times
- Estimated weight of the rhinoceros was 1360kg (3000lbs) with dosage of 0.038mg/kg haloperidol
- Received 50mg (0.037mg/kg) once daily for the first 50 days of treatment
- Dosage increased to 80 mg (0.058mg/kg) once daily for 153 days
- Dose was tapered for the last 34 days to discontinue treatment
- Another female Indian rhinoceros housed at the Cinicinnati Zoo did not receive haloperidol treatment
  - Control for background urinary haloperidol concentrations
Urinary Cortisol

X - transport

Graph showing cortisol levels over time with an X indicating transport.
Urinary Cortisol Comparisons

*different superscripts indicate statistical significance $P<0.05$ within each category (whole, baseline, elevated)
- Positive correlation between EC and cortisol:
  - Urinary EC and cortisol Correlation Coefficient = 0.163 (P < 0.05)
- Not exhibiting normal estrous cycles
- Lack of cycles during time of year when out on exhibit
- First normal ovulatory cycle April 2012
- Otherwise, cystic follicles associated with long follicular phase >14 days
Haloperidol Assay Validation

- B/Bo vs. ng/mL
- Graph shows the comparison between Standard and Pooled urine at various concentrations.
- The x-axis represents ng/mL, ranging from 0 to 5 ng/mL.
- The y-axis represents B/Bo, ranging from 0 to 1.

Legend:
- Standard
- Pooled urine
There were no differences ($P=0.16$) in background concentrations ($0.76 \pm 0.01$ ng/mg Crt; $0.13 \pm 0.01$ ng/mL) of haloperidol between Indian rhinos, and both were similar to background values reported in equine urine ($<0.18$ ng/mL).
A dose dependent excretion effect was observed during dosage decline and concentrations returned to background levels within 2 weeks of treatment ending.
Zoo Volunteer Watch

- 2 hour period: 10am – 12pm daily
- 10 day baseline behavior and exhibit use
- Nikki: 6 day baseline behavior data for comparison of exhibit use and activity (control)
- Change of plan in study design – Manjula off exhibit for 21 days due to need to modify exhibit posts/hot wire

Ethogram for Indian Rhino Manjula
10:00 AM to 12:00 PM on ______/_____/2012

Activity | 10:00 | 11:00 | 12:00 | 1:00 | 2:00 | 3:00 | 4:00 | 5:00 | 6:00 | 7:00 | 8:00 | 9:00 | 10:00
---|---|---|---|---|---|---|---|---|---|---|---|---|---

Comments:

Observer:

Reviewer:

Revises:

[Image of a rhino]
Activity levels

Average Number of Times for Activity over 2-hours

- Charging
- Defecating
- Eating
- Horn rubbing
- Locomoting
- Lying down
- Out of view
- Pool usage
- Standing idle
- Standing up
- Urinating
- Vocalizing

Nikki  Manjula
Pool usage over 2 hour period

- off exhibit
- night access

Dates:
- 05/20/08
- 05/27/08
- 06/03/08
- 06/10/08
- 06/17/08
- 06/24/08
- 07/01/08
- 07/08/08
- 07/15/08
- 07/22/08
- 07/29/08
- 08/05/08
Conclusions

- This is the first data with regard to urinary pharmacokinetics/pharmacodynamics of the LAN haloperidol in the Indian rhinoceros
- No extrapyramidal side effects during 240 days of treatment
Conclusions

- Haloperidol may be useful in:
  - Improving welfare of Indian rhinos or other animals exhibiting difficulty adjusting to new exhibits
- Haloperidol did not appear to interfere with estrous cycle and ovulation
Acknowledgements

- Procter and Gamble Pet Care
- Dr. Monica Stoops, Cincinnati Zoo
- Dr. Carlos Pinto, The Ohio State University
- Veldt Keepers
- Vet Staff
- Kate MacKinnon
- Pat Hermes
Questions?
Current studies on molecular mechanisms of iron homeostasis in rhinoceroses

Rose Linzmeier, Donald E. Paglia, Elizabeta Nemeth and Tomas Ganz
UCLA School of Medicine
Ryan Thompson, Sarah LaMere and Pauline Lee
The Scripps Research Institute, La Jolla
Iron overload in captivity correlates with wild forage
Browsers (shrubs, branches) vs. Grazers (grasses)

Affected by iron overload in captivity

Unaffected
Iron overload in black rhinos
Perls stain iron deposits in tissues

Iron overload in black rhinos
Liver iron levels increase with time in captivity

Zoo Biology (2005) 24:51-72

White

Iron, ug/g wet weight

Age in years

p = 0.007
r² = 0.34
n = 20
Erythrocyte abnormality: Hemolytic anemia in black rhinos

- Hemolysis of RBCs contributes to iron overload
- Can lead to death
- Potential cause: genetic mutation
  - Fragile RBC membrane
  - Prone to lysis

Hemolytic anemia horse
http://www.vetnext.com/
Erythrocyte abnormality: ATP in black rhinos 5% of that in humans

- Anion-exchange HPLC extract red blood cells
  - human (A)
  - black rhino (B)
- ATP required to maintain cell barrier
  - Low ATP levels might contribute to hemolytic anemia

Search for genetic differences related to iron overload

- White vs. Black rhino
- Sequencing mRNA
  - Liver mRNA
    - Iron homeostasis
  - Spleen mRNA
    - Recycling RBCs
- Acquire sequences
- Assemble
  - Trinity software

cmb.molgen.mpg.de/2ndGenerationSequencing
Identify potentially deleterious mutations
SIFT sorting intolerant from tolerant substitutions

Input query: translated RNA sequences

Align related proteins From NCBI database

conserved highly conserved
unconserved

SIFT calculates conservation value at each amino acid position

SIFT predicts effect of substitution at a particular position

tolerated

deleterious

3 candidate mutations identified in black rhinos

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein Function</th>
<th>Link to Black Rhino Phenotype</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC28a2</td>
<td>Solute carrier family 28 member 2 sodium-coupled nucleoside transporter for adenosine</td>
<td>Very low levels of erythrocyte ATP</td>
<td>Q173K Q – Glutamine K – Lysine</td>
</tr>
<tr>
<td>EPB41</td>
<td>Protein 4.1; structural element of erythrocyte membrane skeleton</td>
<td>Hemolytic anemia</td>
<td>G111E G – Glycine E – Glutamic acid</td>
</tr>
<tr>
<td>STEAP4</td>
<td>Six-transmembrane epithelial antigen of the prostate protein family obesity related insulin resistance and inflammatory processes</td>
<td>Suggested link elevated iron stores and insulin resistance</td>
<td>I433S I – Isoleucine S – Serine</td>
</tr>
</tbody>
</table>
**SLC28a2 Q173K**

- ClustalW2 alignment
- Portion of SLC28a2 protein with amino acid position 173
  - Sequence from 36 different species
  - The glutamine (Q) at position 173 invariant
    - Except in black rhino
    - Replaced by lysine
Position SLC28a2 black rhino mutation

Slc28a2 Q173K in transmembrane 1 domain might affect membrane expression

Mol Aspects Med (2013) 34:529-47
Protein 4.1, a component of the erythrocyte membrane skeleton

• Stabilizes erythrocyte shape and membrane mechanical properties, such as deformability and stability

• In humans, rare deletions cause complete loss of protein 4.1R, severe hemolytic anemia
  – A disease common in captive Black rhinos

• Knock-out mouse model
  – Decreased deformability of erythrocyte plasma membrane, increased hemolysis leading to hemolytic anemia
**EPB41 G111E**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Charge</th>
<th># Spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>glutamic acid</td>
<td>acid</td>
</tr>
<tr>
<td>G</td>
<td>glycine</td>
<td>neutral</td>
</tr>
<tr>
<td>R</td>
<td>arginine</td>
<td>basic</td>
</tr>
</tbody>
</table>

Not a conservative substitution
Black rhino only acidic side chain
Position EPB41 black rhino mutation

G111E near start site for erythroblasts translation
Might affect translation initiation

STEAP4 – member of six-transmembrane epithelial antigen of the prostate protein family

- Associated with obesity, insulin resistance, inflammation
  - K/O mouse has metabolic syndrome
  - Related to described black rhino issues

- High expression in adipose tissue
  - In captivity rhinos have greater fat stores

- N-terminal domain has oxidase activity
  - Allow cellular uptake of iron and copper
    - Both essential for glucose and lipid metabolism
Black: GGKRFLS PSLWYPSA YVIALIIPCTVLV SKFILILPC I DRTLTRI RQGW ER
Sumatran: GGKRFLS PSLWYPSA YVIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
Indian: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
White: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
horse: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
platypus: GGKRFLS PSLWYPSA YVLS LILPC VFLV KFILILPC I DRTLTRI RQGW ER
gibbon: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
gorilla: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
chimp: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
human: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
rheus: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
baboon: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
squirrel_monkey: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
marmoset: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
elephant: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
manatee: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
lizard: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
mole_rat: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
bushbaby: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
tree_shrew: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
walrus: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
panda: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
dog: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
cat: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
dolphin: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
orca: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
sheep: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
cow: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
yak: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
frog: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
clawed_frog: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
opossum: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
mallard: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
mouse: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
armadillo: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
bat: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
pufferfish: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
ricefish: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
tilapia: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
rabbit: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
turkey: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
chicken: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
finch: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
guinea_pig: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
pig: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
rat: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER
hamster: GGKRFLS PSLWYPSA YLPS AY VIALIIPCTVLV KFILILPC I DRTLTRI RQGW ER

Amino acids

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>Serine</td>
</tr>
<tr>
<td>I</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>L</td>
<td>Leucine</td>
</tr>
<tr>
<td>V</td>
<td>Valine</td>
</tr>
<tr>
<td>M</td>
<td>Methionine</td>
</tr>
</tbody>
</table>

Not a conservative substitution
Position STEAP4 black rhino mutation

- STEAP4 I433S located in the $\alpha_7$ helix
  - Near site oxidation activity
  - A functionally significant location
- A defect in STEAP4 might explain insulin resistance in black rhinos
- In humans, metabolic syndrome causes mild iron overload

STEAP4 structure:
J Biol Chem (2013) **288**:20668-82

Black rhino insulin resistance:
Conclusions and future plans

- Novel genetic techniques identify causes of hereditary disease
  - SLC28a2, EPB41 and STEAP4
  - Mutations are probably deleterious and located in functionally significant portions of the proteins
- Characterize candidate mutations
  - Express altered proteins and assay their function
- Expand to other affected rhino populations
  - Sumatran rhino tissue for mRNA isolation
    - RNA sequencing and SIFT
    - Identify and analyze candidate mutations
- Understanding the affect of these mutations could lead to improved care and treatment of iron overload in captive black rhinos
Acknowledgements

• Tom Ganz and Ella Nemeth
  – Helpful discussion and direction

• Don Paglia
  – Directing sample collection and insight into iron overload in rhinoceroses

• Pauline Lee
  – Directing RNA sequencing and SIFT analysis

• Ryan Thompson and Sarah LaMere
  – RNA sequencing and SIFT analysis

• Beto Palacios and Damond Ng
  – PCR and DNA sequencing
ISSUE OF CAPTIVE ELEPHANT HEALTH CARE MANAGEMENT IN MYANMA TIMBER ENTERPRISE, MYANMAR

Dr. Zaw Min Oo
Assistant Manager (Vet)
Myanmar Timber Enterprise, Extraction Department, Yangon, Myanmar
INTRODUCTION

MTE, Myanmar Timber Enterprise is one of governmental department under the Ministry of Environmental Conservation and Forestry (MOECAF).

Before independent, The name of MTE was Stated that Timber Extraction Organization (TEO) At 1948, changed to State Timber Board (STB). At 1972, changed again from STB to Timber Corporation. Finally, in 1989, changed again from Timber Corporation to Myanmar Timber Enterprise (MTE) from Timber Corporation.
In MTE, there are eight main departments. These are:-

- Planning and Statistic
- Extraction
- Engineering
- Financial
- Saw mill
- Timber export and import
- Timber local used
- Wood based Industrial Department
Staff population

- In MTE, there are 11221 staff only in Extraction including elephants’ staff (Chief Mahouts, Leaders and elephant riders) and other staff (Officers, Range officers and Veterinarians)

- All over country, there are 41 vets in MTE.

- They are only responsibilities for MTE elephants, not for private and wild elephants.
**Elephant population in MTE**

**Total Elephant population**

<table>
<thead>
<tr>
<th>Region</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>In MTE</td>
<td>2861</td>
</tr>
<tr>
<td>In Private</td>
<td>&lt; 2000</td>
</tr>
</tbody>
</table>
Distribution of wild elephant in Myanmar

Wild elephant population (Estimate) 4000-6000 (1997)

- Sagaing Region  ≈ 250
- Chin State       ≈ 350
- Mandalay        ≈ 100
- Magwe           ≈ 100
- Rakine          ≈ 400
- Bago            ≈ 300
- Ayeyarwaddy     ≈ 350
- Kayin and Tanintharyi ≈ 250

Totally, estimated population > 3000
Death and Birth rate of elephants in MTE

In 2010 -2011

Birth rate 2.6%
Death rate 2.7%
Yearly
Causes of death in MTE, based on 2012-13 budget year

- Old aging: 26%
- Accidental cases: 5%
- Snake bite: 8%
- Bloat: 3%
- Elephant Attack: 6%
- Constipation: 5%
- Anemia, Malnutrition: 2%
- Viral Disease: 15%
- Still birth: 3%
- Respiratory D/s: 6%
- Distocia: 2%
- Diarrhoea: 8%
- Heat Stroke: 2%
- Unknown: 11%
- To be confirm???
Elephant Registration and Identification
Elephant Identification or Registration by Tattooing
Elephant Health Care Management

Elephant camp composition (Just brief;)

- Each camp with at least 5 or 6 elephants (2 males and 4 females)
- With 12 mahouts
- Not more than 1200 tonnage per working seasons with 6 working elephants (5 or 6 elephants)
Common Health problems in MTE

- Mostly work-related injuries such as broken legs or fracture, abscess, fibrosis, hill Clift (Improper Management)
- Malnutrition
- Parasitic infestation
- Infectious diseases
- Eye problems
- Accidents
In Abdominal Area

In Leg
Surgical removing and suturing
Eye Problems
Wound on the foot and foot Pad
Accidental Injuries
Parasitic problem
Prevention for parasite

- As a parasitic prevention, use the following drugs:
  - Albendazole tablets
  - Ivermectin 1% SC or IM
Prevention for Some Infectious Diseases

HS vaccine

Anthrax spore vaccine

Need serological research????
Suspected Infectious Disease

EEHV or Other infectious Diseases ???
Baby elephants die with infectious disease ???
On Going Project for Elephant Care managements
In conclusions;

- Numbers of Myanmar captive elephant are gradually decreased.
- Numbers of wild elephant are also gradually decreased in Myanmar.
- Human-elephant conflict cases are still remained in some areas.
- So, we need to do conservation for wild and captive elephants.
- On the other hand, we need to do not only research with elephant but also to up-grade altitude of mahouts. (Mahout welfare)
Acknowledgements

➢ I wish my thinks to authorize persons from IEF for their kindly support and everything for my many request.
➢ I also would like to say special thanks to Dr. Heidi Riddle for her helpfulness of my trip.
Thank you for your attention
Elephant Care Stakeholders Taskforce

3rd Annual Management and Research Priorities of Tuberculosis for Elephants in Human Care

PITTSBURGH  AUGUST 25-26th
Representative Institutions

• AAZV
• IEF
• EMA
• AZA
• Feld

• Group of specialists in fields of elephant husbandry, veterinary medicine and management
  • Ft. Worth 2011
  • Tulsa 2012
  • Pittsburg 2013
Tuberculosis in Elephants USA

- *Mycobacteria tuberculosis* and *M. bovis*
- Asian Elephant
- Incidental reports last century
- Recognized as concern in 1990’s
- Current prevalence in population is low
Tuberculosis Disease

• Most likely originally human to elephant
• Elephant to elephant spread possible
• Infection without clinical signs of illness
• Diagnosis is not straight forward
• Need to use multifactorial approach
  – History of individual and herd
  – Trunk wash culture
  – Serologic screening
  – Molecular based technologies
USAHA Developed Guidelines 2008

- Adopted by USDA
- Elephants segregated into groups
- Based on
  - Exposure
  - Trunk wash culture
  - Serologic testing
  - Travel restrictions
  - Increased testing
What are we doing?

• Industry stakeholders working to answer questions about the diagnosis and treatment of TB in elephants
• Organize and promote research into many unknowns about this disease
• Review and comment to the USDA guidelines for control of TB in elephants
• Organize elephant community to share information and care of affected elephants
What have our members been up to?

• Promoted, funded and identified research topics into the many unknowns about elephant tb
  – IEF funded elephant tb epidemiology survey
  – IEF funded evidence based review of elephant tb diagnostic publications
  – Recently published paper on point prevalence of tb in elephants.

• Developed and submitted comments to the federal register about the USDA’s adoption of the 2010 guidelines

• Developed and submitted comments to the USAHA tb subcommittee about the 2012 guidelines

• Petitioned the USAHA to admit new members to the USAHA elephant tb subcommittee
This Year: Still lots to do

- ECT will continue to promote good science and evidence-based approach to the diagnosis
- New treatment modalities
- Exploring new antigen-based diagnostic tests
- Promote a multipronged approach to TB diagnosis in elephants
- Develop consensus definitions to better describe infection, exposure, and prevalence of the disease.
- Develop stakeholder-based guidelines for the control and treatment of tuberculosis in elephants.
What Next?

• Planning to meet again, St. Louis?
• Continue to identify areas of research into, treatment, diagnostics and epidemiology
• Work to get the information available to stakeholders
• Revise guidelines as new information becomes available
• Collaboration and patience
TESTING FOR TUBERCULOSIS IN ELEPHANTS: WHAT IS THE EVIDENCE?

David Miller, DVM, DACZM, PhD  
Ramiro Isaza, DVM, MS, MPH, DACZM  
Dennis Schmitt, DVM, PhD DACT  
Jared Taylor, DVM, MPH, PhD, DACVIM, DACVPM  
David Claborn, MS, DrPH  
Kay Backues, DVM, DACZM
Overview

- Background

- Systematic review of diagnostic assays for tuberculosis in elephants

- Diagnostic decision-making
Elephant Tuberculosis Challenges

- Bacteria
  - *Mycobacterium tuberculosis*
  - *M. bovis*

- Elephant/zooological species

- Human concerns

- Livestock concerns
Elephant Tuberculosis Challenges

- Clinical - general
  - Respiratory disease
  - Gastrointestinal disease
  - Wasting

- Clinical - elephants
  - Respiratory disease
  - Gastrointestinal disease
  - Wasting
  - No signs
Elephant Controversies

- Conservation
- Elephants in captivity
- Working elephants
- Zoos
- Animal interest groups
- Society

TB
Dx
Tuberculosis Overview

- Diagnosis – The challenge
  - **Post-mortem**
    - **Culture**, Molecular (gene probes)
  - **Ante-mortem**
    - **Trunk wash + culture**
      - Directly identifies *Mycobacterium*
  - **Serology** (blood test) (**STAT-PAK™, MAPIA™, DPP®**)
    - Indirect: identification of immune system response to TB
    - **Limitations - source of controversy**
Resolution of differing opinions

- **Solution**
  - Cochrane Collaboration
  - GRADE (Grades of Recommendation, Assessment, Development and Evaluation)
    - Confidence in clinical guidelines
  - US Public Health Grading System
    - Experimental design and strength of recommendation
  - Internal and External validity

- Not mutually exclusive
- Clarification of points of disagreement
Systematic Review

- Results
  - No data on test characteristics for culture, acid-fast, or cytokines
  - Mikota (2001): Intradermal tuberculin
    - Se: 16.7% (0.9–63.5%)
    - Sp: 74.2% (55.1–87.5%)
  - **Serology**: 17 estimates of 9 assays among 5 studies
Study evaluations – Results

- Clarity:
  - Inconsistent/obtuse reporting of methods & results
    - Space limitations
    - EQUATOR, CONSORT, STARD,......
    - Varying backgrounds, perspectives, personal interests
    - Training

- Scope of inference
  - Definition of population
  - External validity
Systematic Review

- Study evaluations – Results
  - Risks of bias
  - Method quality limitations
  - Experimental design challenges
    - Gold standard flaws
    - Challenges of identifying representative population/spectrum
  - Internal validity: generally low to moderate
  - Limited external inference (external validity)

Concepts ➔ Details
## Systematic Review

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Se(^a) (95% CI)</th>
<th>Sp(^b) (95% CI)</th>
<th>PV+(^c) (95% CI)</th>
<th>PV-(^d) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bontekoning et al, 2009</td>
<td>STAT-PAK</td>
<td>80% (62.47-97.53%)</td>
<td>87.23% (80.49-93.98%)</td>
<td>57.14% (38.81-75.47%)</td>
<td>95.35% (90.90-99.80%)</td>
</tr>
<tr>
<td>Greenwald et al, 2009</td>
<td>STAT-PAK</td>
<td>100% (84.0-100%)</td>
<td>100% (96.0-100%)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Verma-Kumar et al, 2012</td>
<td>STAT-PAK</td>
<td>48.6% (37.2-61.0%)</td>
<td>99.3% (96.7-99.9%)</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Se = test sensitivity = proportion of infected cases that are correctly identified  
Sp = test specificity = proportion of infected cases that are correctly identified  
PV+ = positive predictive value = proportion of test positive that are correct  
PV- = negative predictive value = proportion of test negatives that are correct

**Why the variation?**
All populations are not necessarily alike
- Test performance varies by (sub)population
Experimental design challenges for serological testing for antibodies to tubercular infections in elephants

**Graph A**: 
- **TB Antibody Level**: 0
- **Time**: t = 0 for infection
- **Increasing bacteria number**: Increasing host pathology
- **Anergy?**

**Graph B**: 
- **Number of elephants**: Uninfected elephants
- **Stage of Infection**: Subclinical/early infections
- **Late stage infections**

**Time**: 
- **Early stage infections**
- **Late stage infected elephants**
What is the question: objectives vary

- Detection or exclusion of disorder
- Decisions for diagnostic or therapeutic management
- Monitoring clinical course
- Prognosis
- Measure general health or fitness
Potential for monitoring clinical course

- Lyashchenko, et al 2012
- Serial serology
- “Predictive value”
What is the question: objectives vary

- Detection or exclusion of disorder
- Decisions for diagnostic or therapeutic management
- Monitoring clinical course
- Prognosis
- Measure general health or fitness

- Multiple targets of concern
  - Elephants – individuals & “contacts”
  - Public
  - Occupational health
Concept of analytical vs. clinical test validity

- Confusion: hierarchical assessment of diagnostic test
  - Phase I: Do “sick” and “normal” individuals have different test results?
    - Known diagnosis → diagnostic test
  - Phase II: Do test results correspond to disease likelihood?
    - Se, Sp, PV+, PV-
    - Diagnostic test result → diagnosis
    - Requires full-spectrum of disease or specify subpopulation
  - Phase III: Does test distinguish + & - among suspects?
    - Validity threatened if reference standard is lost, not done, or indeterminate

Haynes & You, 2009
Systematic Review

- Concept of analytical vs. clinical test validity
  - Confusion: hierarchical assessment of diagnostic test
    - Evidence-based clinical decision-making
      - Phase IV: Do patients receiving the test ultimately have better outcomes than patients that don’t?
        - Randomization
      - Phase V: Does use of the diagnostic test lead to better health outcomes at an acceptable cost?
        - Randomization
        - External validity threatened if study subjects differ from those in “real practice”

Haynes & You, 2009
Why does this matter?
Prostate cancer
- #2 cancer in men world-wide
- 6th leading cause of death in men
- More common in 1st degree relatives with prostate cancer
- Rarely has reliable early warning signs
- Usually does not cause clinical signs or symptoms

Clinician perspective: increased vigilance for screening
Is this the correct response?
Screening (PSA) for prostate cancer

- No decrease in mortality
- False-positives
  - Harms – frequent and moderate
    - Minor: bleeding, anxiety,...
    - Major: over-diagnosis and overtreatment, infection, pneumonia
  - Insufficient data available on quality of life
- Harm > benefits

Ilic et al, 2013
Relevance to elephants:

- Emotional attachment
- Un-established testing benefit
  - Risk of false-positives
    - Does the test benefit elephants?
    - Costs?
    - Risks?
Effect of disease prevalence on test accuracy
- False-positives increase as disease prevalence ↓; disease eradication programs’ challenge
- Basic veterinary epidemiology
Effect of disease prevalence on test accuracy

**Diagnostic Test with 95% Sensitivity and 95% Specificity**

- PPV+ and PPV- values for different prevalence rates.

**Diagnostic Test with 80% Sensitivity and 80% Specificity**

- PPV+ and PPV- values for different prevalence rates.

**Diagnostic Test with 60% Sensitivity and 60% Specificity**

- PPV+ and PPV- values for different prevalence rates.

<table>
<thead>
<tr>
<th>True prevalence of disease = 5%</th>
<th>PV+</th>
<th>PV-</th>
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<tbody>
<tr>
<td>Se 95%/Sp 95%</td>
<td>50%</td>
<td>99%</td>
</tr>
<tr>
<td>Se 80%/Sp 80%</td>
<td>17%</td>
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<tr>
<td>Se 60%/Sp 60%</td>
<td>7%</td>
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Diagnostic Decision-Making

Key take home messages:

- Currently available research with limited external validity
- Current research in early phases of test development
- Substantial study design challenges for rigor
- Data is limited for rigorous clinical decision-making
Point prevalence and incidence of Mycobacterium tuberculosis complex in captive elephants in the United States of America

Ramiro Isaza, DVM, MPH, DACZM

Zoological Medicine Service
College of Veterinary Medicine
University Florida
SHORT COMMUNICATION

Point prevalence and incidence of *Mycobacterium tuberculosis* complex in captive elephants in the United States of America

Melissa Feldman\(^a\), Ramiro Isaza\(^b\)*, Cindy Prins\(^c\) and Jorge Hernandez\(^d\)

\(^a\)Department of Health Services Research, Management, and Policy, University of Florida, Gainesville, USA; \(^b\)Department of Small Animal Clinical Sciences, University of Florida, Gainesville, USA; \(^c\)Department of Epidemiology, University of Florida, Gainesville, USA; \(^d\)Department of Large Animal Clinical Sciences, University of Florida, Gainesville, USA

(Received 2 December 2012; final version received 30 January 2013)

**Background:** Captive elephants infected with tuberculosis are implicated as an occupational source of zoonotic tuberculosis. However, accurate estimates of prevalence and incidence of elephant tuberculosis from well-defined captive populations are lacking in the literature. Studies published in recent years contain a wide range of prevalence estimates calculated from summary data. Incidence estimates of elephant tuberculosis in captive elephants are not available.

**Objective:** This study estimated the annual point prevalence, annual incidence, cumulative incidence, and incidence density of tuberculosis in captive elephants within the USA during the past 52 years.

**Animals and methods:** We combined existing elephant census records from captive elephants in the USA with tuberculosis culture results obtained from trunk washes or at necropsy. This data set included 15 years where each elephant was screened annually.

**Results:** Between 1960 and 1996, the annual point prevalence of tuberculosis complex mycobacteria for both species was 0. From 1997 through 2011, the median point prevalence within the Asian elephant population was 5.1%, with a range from 0.3% to 6.7%. The incidence density was 9.7 cases/1000 elephant years (95% CI: 7.0–13.4). In contrast, the annual point prevalence during the same time period within the African elephant population remained 0 and the incidence density was 1.5 cases/1000 elephant years (95% CI: 0.7–4.0).

**Conclusions:** The apparent increase in new cases noted after 1996 resulted from a combination of both index cases and the initiation of mandatory annual tuberculosis screening in 1997 for all the elephants. This study found lower annual point prevalence estimates than previously reported in the literature. These discrepancies in prevalence estimates are primarily due to differences in terminology and calculation methods. Using the same intensive testing regime, the incidence of tuberculosis differed significantly between Asian and African elephants.

**Clinical importance:** Accurate and species specific knowledge of prevalence and incidence will inform our efforts to mitigate occupational risks associated with captive elephants in the USA.

**Keywords:** Mycobacterium tuberculosis; Elephas maximus; Loxodonta africana; prevalence; incidence
# Basic Elephant Statistics

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Basic Elephant Statistics
Basic Elephant Statistics
Introduction

Prevalence of *M. tuberculosis* (*M. tb*)

- Is the prevalence really increasing?
  - Increased TW culture testing since 1997
  - Is there a difference between species
  - Revisit calculations of prevalence
    - "Prevalence" is not total cases / current population
Introduction

Measures of *M. tb* complex for both species of elephants

◆ What is the annual point prevalence?
◆ What is the annual incidence?
◆ What is the incidence density?
Introduction

Mandatory math review

\[ \frac{3}{4} \text{ Numerator} = \text{Elephant M. tb “Cases”} \]

\[ = \text{Elephant Population} * \]

* An “open” population, with births and deaths, changes with time
Elephant Cases

Elephant cases of “M. tb complex”

- Verified culture of M. tb or M. bovis
- Documented within the SSP population
- Present at the time of counting
- Each case only counted “once”
- Cases can not be “removed”
Elephant Cases

Elephant cases of “M. tb complex”

◆ 50 total new cases (1960-2011)

- Asian elephants (45 M. tb)
- African elephants (4 M. tb)
  (1 M. bovis)
Study Elephant Population

- Captive elephants living in the USA and in the SSP Population
  - Tabulated from SSP data for any given point in time

For example: Today’s population

- 224 Asian
- 193 African
Methods for Prevalence

• Point prevalence of *M. tb* complex from 1960 to 2011
  – Calculated on the first day of each year

\[
\frac{\text{# of Live Elephants Culture Positive for TB}}{\text{Total Population of Live Elephants}} = \text{Point Prevalence}
\]
M.t.b Complex in Asian Elephants

PREVALENCE OF TUBERCULOSIS IN ASIAN ELEPHANTS

Prevalence (%)

Year

**M. tb** Complex in *Asian* Elephants

**Prevalence of M. tb complex**

- Median since 1997 = 5.0%
- Range = 1.2 – 5.8%
**M. tb** Complex in **Asian Elephants**

**Prevalence of M. tb complex**

- Median since 1997 = 5.0%
- Range = 1.2 – 5.8%

- Current (today) = 20/224 = 8.9%
M. *tb* Complex in **African** Elephants

**PREVALENCE OF TUBERCULOSIS IN AFRICAN ELEPHANTS**

![Graph showing prevalence of tuberculosis in African elephants over time.](image-url)
Methods for Incidence

• Incidence of elephants culture positive for *M. tb* complex from 1960 to 2011
  – Calculated for each year

\[
\text{Annual Incidence} = \frac{\text{Number of new cases in one year}}{\text{Average population during that year}}
\]
M. tb Complex in Asian Elephants
**M. tb** Complex in **African** Elephants

![Graph showing new cases of tuberculosis in African elephants]

**NEW CASES OF TUBERCULOSIS IN AFRICAN ELEPHANTS**

- **Year**
  - 1960
  - 1970
  - 1980
  - 1990
  - 2000
  - 2010

- **Total New Cases**
  - 0
  - 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10

The graph indicates a significant increase in the number of new cases starting from 2000.
Methods for Incidence Density

- Incidence density of elephants culture positive for *M. tb* complex in the US
  - Calculated between 1997 and 2011 when the whole population was aggressively tested every year

\[
\frac{\text{Number of new cases in a period of time}}{\text{Number of “elephant years” at risk}} = \text{Incidence Density}
\]
Asian Elephant Incidence Density

Incidence Density of *M. tb* complex

- Annual incidence density between 1997 and 2011
  - 9.7 cases /1000 elephant years
    (95%:7-13) since 1998
Incidence Density of *M. tb* complex

- Annual incidence density between 1997 and 2011
  - 1.5 cases /1000 elephant years
    (95%:0.7-4) since 1998
Conclusions

**M. tb complex in elephants**
- Need accurate census data
- Need well defined cases and populations
- Need consistent calculations
Conclusions

_M. tb_ complex in elephants

◆ What does it all mean?

◆ Asian elephants average about 5% prevalence
  - Current estimate is 8.9%
◆ About 2-4 new cases each year
◆ 9.7 cases for every 1000 elephants screened
◆ Very significant difference between species
FIRST EVIDENCE OF EEHV INFECTION IN SUMATRAN ELEPHANTS (*Elephas maximus sumatranus*) IN INDONESIA

Christopher Stremme 1), Arun Zachariah 2), Adin Priadi 3)

1) Veterinary Society for Sumatran Wildlife Conservation, Medan, North Sumatra / Indonesia
2) Department of Forests and Wildlife, Government of Kerala, Sultan Battery, Wayanad / India
3) PT Satwa Duta Medical Laboratory for Animal Health, Bogor / Indonesia
1. Case Kristina

- Captive born, mother captive (wild caught), father wild
- Born 17\textsuperscript{th} September 2003, naturally raised by mother
- Kept in a group consisting of 2,2; regular access to wild elephant habitat
- Neither this elephant itself nor any other elephant that it has been in contact with ever had contact with African elephants.
- Never any visible health problems,
- Routine deworming's every 3 - 4 months with ivermectin or albendazol
1. Case, Kristina

In the evening of the 21.2.2006 mild anorexia and some very mild ataxia symptoms disappeared soon without treatment.

In the afternoon of 22.2.2006 again anorexia, drowsiness, and some mild ataxia observed, died few hours later on the same evening.
MAJOR GROSS PATHOLOGICAL LESIONS
ORGAN SAMPLES STORED ON ICE AND IN 96% ETHANOL WERE TAKEN

NO CASE LIKE THIS HAD SO FAR BEEN REPORTED IN SUMATRA

LIMITED LAB FACILITIES

TESTED AT A LOCAL LIVESTOCK LAB FOR:
HEMORRHAGIC SEPTICEMIA (PASTEURELLA MULTICODA) => NEGATIVE
E. COLI => POSTIVE BUT NO EXAMINATION FOR ENTERO-PATHOGENICITY / TOXICITY => E.COLI ENTEROTOXAEAMIA COULD NOT BE DIAGNOSED

NO LAB FACILITIES FOR EEEHV AVAILABLE IN INDONESIA AT THAT TIME

NO SAMPLES OF THIS CASE WERE STORED

THE MOTHER GAVE BIRTH AGAIN ON THE 29th JUNE 2008 TO A MALE CALF, NATURALY RAISED, STILL ALIVE
WART LIKE LESIONS FOUND IN OTHERWISE HEALTHY, JUVENILE SUMATRAN ELEPHANTS
BLISTERS AND SMALL LESIONS FOUND ON THE MOUTH MUCOSA IN OTHERWISE HEALTHY SUMATRAN ELEPHANTS
2\textsuperscript{nd} AND 3\textsuperscript{rd} CASE, NAMO AND TANGKA

- Both captive born;
- mothers and father of both are captive but wild caught as juveniles from the same family group -> most likely genetically closely related
- Kept in a group consisting of 1,6; access to wild elephant habitat.
- Neither these two elephants themselves, nor any other elephant they had been in contact with, had contact with African elephants
2ND CASE, NAMO

*NAMO* born 6\textsuperscript{th} November 2010, no complication during birth process, naturally raised; never any visible health problems, from an age of 6 months on, regular antiparasitic treatment with ivermectin and albendazole every 4 months, basic training initiated at 6 months of age.
2ND CASE, NAMO

On the 3rd of April 2012 conducting routine deworming (ivermectin 0.15mg 7 kg BW p.o.)

On the morning of the 5th of April 2012 the calf is found dead, no abnormalities had been observed by the mahouts during the previous day and the evening bathing and feeding procedures.
2ND CASE, NAMO  MAJOR GROSS PATHOLOGICAL LESIONS
3rd Case, Tangka

Tangka born 14th September 2010, no complication during birth process, naturally raised; from the age of about 7 months on, a few times developing some intestinal problems such as constipation and mild diarrhea, these problems usually resolved without any treatment after 1 to 3 days, from an age of 6 months on, regular antiparasitic treatment with ivermectin and albendazole every 4 months, basic training initiated at 5 months of age.
On the 3rd of April 2012 conducted routine deworming (ivermectin 0,15mg / kg BW p.o.)

No signs of discomfort or illness is observed during the following days. Despite the normal health appearance started prophylactic treatment with (15mg/kg BW) Famciclovir on the 7th of April.

In the evening of the 10th of April 2012 mild discomfort, anorexia and swelling of the lower jaw is observed, the calf is still normally nursing. The calf goes in recumbent position in the early morning and dies 2,5 hours later.
3\textsuperscript{ND} CASE, TANGKA  MAJOR GROSS PATHOLOGICAL LESIONS
POSTMORTEM

Two sets of tissue samples from all organs with pathological changes were collected and preserved in 96% ethanol and deep frozen at -20°C.

Due to a lack of diagnostics for EEHV in laboratories in Indonesia the samples could not be tested immediately.

About 6 months after collection of the samples a specific laboratory facility for molecular diagnosis of EEHV was established in Bogor.
EEHV DIAGNOSTIC

DNA WAS EXTRACTED FROM BOTH FROZEN AND ALCOHOL PRESERVED SAMPLES FROM HEART, SPLEEN AND LIVER.

BOTH CASES WERE IDENTIFIED AS EEHV 1 BY CONVENTIONAL DIAGNOSTIC PCR FOR PAN-EEHV POL AND EEHV1-SPECIFIC POL.

THESE WERE THEN SUBJECTED TO DETAILED GENE SUBTYPE DNA SEQUENCING AT THREE KEY PCR LOCI, U38/POL, U51/VGPCR, U60/TER.

THESE TWO CASES HAVE IDENTICAL EEHV1A DNA SEQUENCES TO ONE ANOTHER INDICATING A COMMON EPIDEMIOLOGICAL SOURCE.
SOME CONCLUSIONS AND QUESTIONS FROM THE CASES

- EEHV IS PRESENT IN SUMATRAN ELEPHANTS IN INDONESIA
- POST MORTEM SAMPLES CAN BE STORED AND PRESERVED DEEP FROZEN AND IN 96% ETHANOL FOR OVER & MONTHS
- WHAT HAS TRIGGERED THE OUTBREAK OF THE LETHAL DISEASE???
- COULD THE GENETIC CLOSE RELATION OF THE PARENTS BE A PRE-DISPOSITIONING FACTOR
I thank Elephant Family, Benindi Fund, AES and the US Fish and Wildlife Service for their funding support helping to enable Vesswic’s veterinary works.

I thank the national and provincial Indonesian Nature conservation Agencies (PHKA and BKSDA), the camp managements and mahouts for the good collaboration.

I thank the Pittsburgh Zoo & PPG Aquarium for inviting me to this symposium and present some of our works in Sumatra.
THANK YOU

FOR YOUR ATTENTION
SEVEN SPECIES OF ELEPHANT ENDOTHELIOTROPHIC HERPESVIRUSES (EEHVs) FORM A NOVEL MAMMALIAN SUB-FAMILY DESIGNATED THE DELTAHERPESVIRINAE.

*Hayward GS¹, Zong J-C¹, Heaggans SY¹, Long SY¹, Pearson VR², Lock J.³, Schneider R³, Latimer EM³, Reid JG⁴, Qin X⁴, Muzny DM⁴, Gibbs R⁴, Peng R-S⁵, Petrosino J⁴ and Ling, PD⁵.

¹Viral Oncology Program, The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins School of Medicine, Baltimore, MD 21231; ²Department of Molecular Biology, Princeton University; ³National Elephant Herpesvirus Laboratory and Pathology Department, National Zoological Park, Washington, DC.; ⁴Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030; ⁵Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030, USA.

Nearly 90 suspected cases of lethal EEHV hemorrhagic disease plus ten more survivors of high load symptomatic EEHV viremia have been reported worldwide, primarily in young Asian elephants, including 37 in North America, 28 in Europe and over 30 in Asia [Zong & Hayward, 2011; Hayward, 2012]. At least 57 of these cases have been confirmed and compared by PCR based “DNA fingerprint” gene sequencing. Another 30 EEHV strains have been identified from trunk wash or saliva shedding in asymptomatic zoo elephants, as well from either biopsied skin nodules or necropsied lung nodules from healthy African elephants [Zong J-C et al; Pearson VR et al. MS in preparation]. The results of extensive PCR genotype sequencing analysis of most of these samples have revealed seven distinct EEHV species [Latimer et al, 2011] and several additional chimeric sub-species that have diverged over the entire 100 million year evolutionary history of the Afrotheria, the ancestors of modern elephants [Fig 1]. These and other recent results from samples obtained from Asian hemorrhagic disease cases in range countries [Zachariah et al, 2013] have also now greatly clarified both the origins and epidemiology of this disease, as well as the evolutionary relationship between the Proboscivirus genus and other mammalian herpesviruses.

In particular, the complete 177,136-bp genome of a strain of the most highly pathogenic species EEHV1A(Kimba) has been compiled by next generation sequencing techniques on DNA extracted directly from infected Asian elephant necropsy liver tissue [Ling et al, 2013]. From a total of 22 gigabases of raw Illumina sequence data a subset of sequence runs at a relatively high abundance of between 20 to 35 copies per cell were filtered out and assembled by de novo bioinformatics approaches without the use of a reference sequence. This yielded a pool of 350 unlinked contigs in all, but with one intact block of 83-kb corresponding closely to the core segment that we had already obtained by PCR walking approaches. Another five of the largest contigs were then joined together with this larger segment by filling in gaps across the ends by PCR amplification yielding a new 162-kb super contig. Two further approaches were then employed to attempt to identify any remaining additional smaller viral contigs. The first used a manual elimination screening process to remove all contigs from the pool that appeared to be cellular, based on the presence of homology to high copy number mammalian sequences, the presence of repetitive telomere-and sub-telomere like sequences or the presence of high pyrimidine-purine tract biases. This process left 11 more potential viral contigs adding up to nearly 15-kb in size (including several with small CA-repeat indels of 15-30-bp in length), which all then proved to be present by PCR in six other infected Asian elephant DNA samples tested, but not in any of six uninfected Asian elephant DNA samples. A second approach involved carrying out a long-range PCR amplification experiment in the outwards directions with primers from the two ends of the 162-kb contig, which yielded a single PCR product of 15.8-kb in size representing an apparent join across a closed circular or palindromic intracellular form of the viral genome. Remarkably, when this PCR “end-join” product was
sequenced all 11 of the small remaining “candidate” contigs above proved to be present within it with only small gaps between them. Subsequently, nearly the entire novel segment was also re-confirmed by standard Sanger PCR sequencing to correct about 50 frame-shift and other errors in the Illumina generated data. Sequence data and annotation for the final complete EEHV1A(Kimba) genome is available online at NCBI Genbank Accession No KC618527.

**Figure 1:** Summary diagram illustrating the genetic relationships between individual EEHV species, including the EEHV1B chimeric sub-species (#). The dendrogram on the left-hand side indicates genetic divergence values as average nucleotide differences (%) across sequenced portions of these genomes. GC-rich and AT-rich branches refer to overall G-plus-C content of the DNA. Red boxes indicate natural host Elephas maximus. Green boxes indicate natural host Loxodonta africana. The number of confirmed lethal cases associated with each virus species compared to the total number of examples identified so far for each species, together with the year of original discovery are listed on the right-hand-side.

Overall, the data revealed that whilst 51 of the total of 115 genes have orthologues in other herpesviruses, the other 64 are novel, including 23 members of the 7xTM rhodopsin-vGPCR family (eight of which resemble chemokine receptors), ten immunoglobulin family (IgFam) genes and two glycosyl tranferase enzymes (vGCNT1, vFUT9) that all represent “captured” or “pirated” cellular genes. The IgFam includes three versions of cellular vOX2(CD200) genes, including a
duplicated spliced pair of “old” very anciently acquired and now highly diverged genes (vOX2-2 and vOX2-3), each with about 25% residual identity to the mammalian versions, as well as another “new” more recently acquired unspliced vOX2-1 gene that encodes a protein with 98% identity over 280 amino acids within the functional domains in exon3 plus exon4 to the host Elephas, Loxodonta and Mammuthus versions of this protein. Nevertheless, since it is also present in the EEHV2, EEHV5 and EEHV6 genomes and has diverged by 20% at the DNA level from the elephantid host versions, the “new” vOX2-1 gene (despite having not changed significantly at the protein level) must have been captured some 20 million years ago and have been under high selective pressure not to diverge throughout that entire time period. The captured vGCNT1 protein has 69% identity to the equine version and the vFUT9 has 52% amino acid identity to all mammalian versions.

Figure 2: Radial DNA level phylogenetic tree for the intact 2,500-bp DNA polymerase (U38/POL) genes from five species of EEHVs (= Deltaherpesviruses) compared to their orthologues in key representative herpesvirus species from all three other mammalian subfamilies (Alpha, Beta and Gamma). The dendrogram was generated in Mega5 after clustal alignments with the program Muscle.
Incomplete PCR-based DNA sequence analysis from another two strains each of EEHV1A, EEHV1B and EEHV2 (totaling 60 to 80-kb each), as well as three strains of EEHV5 and two of EEHV6 (totaling 25 to 30-kb each) has shown that all five AT-rich branch EEHV species (which diverge from each other by 15 to 20% at the nucleotide level) have an inversion of a large 40-kb core segment of the genome relative to all mammalian betaherpesviruses. Three other species EEHV3, EEHV4 and EEHV7 (just 4-kb each) are even more highly diverged (35%) and form a distinct GC-rich branch of the Proboscivirus genus [Fig 1]. The AT-rich branch EEHVs also all encode alphaherpesvirus-like thymidine kinase (TK), ribonucleotide B-subunit (RRB) and UL9-like origin-binding protein (OBP) genes plus a dyad symmetry Ori-Lyt domain that are absent.
from beta-herpesviruses. They also encode orthologues of the conserved protein kinase (CPK) enzyme that could potentially target the anti-viral drugs FCV or GCV. Most dramatically, all EEHV genes and proteins encoded in common with other herpesviruses are at least 50 to 80% diverged from their nearest orthologues. In both DNA and protein based phylogenetic trees the EEHVs fall into a monophyletic clade branching intermediate between the mammalian gammaherpesvirus and beta-herpesvirus sub-families [Fig 2]. Because of all these novel features, we propose that the Probosciviruses (= EEHVs) should be designated as the prototypes of a new Deltaherpesvirinae sub-family, which we estimate has evolved separately from the three other mammalian Herpesviridae sub-families within Afrotherian hosts, including the ancestors of modern elephants, for more than 100 million years.

Many EEHV species also exhibit multiple chimeric features and subtype variants [Zachariah, et al 2013]. In particular, all well characterized EEHV1 strains fall into two sub-species clusters called EEHV1A and EEHV1B that differ overall by 4.5% at the nucleotide level. But this divergence is not uniform. Instead they have three major chimeric domains (totalling 10-kb) within the conserved core herpesvirus gene block that are highly diverged [domains I, II and III in Fig 3]. These encompass part or all of 14 genes, including gB-POL (3.0-kb), ORFJ-gN-gO-gH-TK (3.8-kb, 32%) and ORFM-ORFN-UDG-gL-ORFP-ORFQ (6.5-kb) that differ between the two sub-species by 17, 32 and 30% at the DNA level. All six evaluated strains of EEHV1B have linked versions of these three chimeric domains in common and show up to ten-fold less overall genetic variation amongst them than do the EEHV1A strains.

The remainder of the EEHV1B genome displays additional mosaic features including large pieces that are almost indistinguishable from of a modern EEHV1A genome, combined with an older EEHV1A segment of 25-kb that has diverged by between 2-3%. These are joined to the three chimeric domains that were evidently derived from one or more other related but unknown EEHV viruses that had diverged considerably further than the 15% that EEHV1A has from EEHV6. In addition to all of that, several other EEHV1 genes such as glycoprotein-H(gH) in [domain II], vGPCR1, vGPCR5, vGPCR6, vOX2-2 and vOX2-3 [domain V] all cluster into three to five hypervariable sub-types each. Finally, a set of between eight and 14 adjacent genes on the far right-hand-side [domain IV in Fig 3], including vFUT9 (with two subtypes that are 44% diverged), as well as vGPCR8 and five vIgFam genes, are also highly variable and form multiple distinct subtypes that are partially unlinked from the 1A versus 1B sub-species patterns. Most unusually, several of the latter have either been deleted in a sub-set of EEHV1 strains or sometimes instead have “fragmented” versions carrying multiple “out-of-frame” mutations. Overall, we conclude that the vast majority of the numerous lethal and non-lethal EEHV1 strains found worldwide last had common ancestors in the time-frame of between many hundreds of thousands of years ago up to several million years ago. These viruses must have been very common and wide-spread infections within wild Asian elephant populations for at least that length of time. Both EEHV1A and EEHV1B strains have been found in lethal hemorrhagic disease cases in America, Europe and Asia, and many elephant housing facilities have had cases of hemorrhagic viremic disease or identified asymptomatic shedding associated with both EEHV1A and EEHV1B [Stanton et al 2011]. Furthermore, five closely monitored surviving healthy Asian elephants in America have been observed to become infected sequentially with either EEHV1A and then EEHV1B or vice versa [Stanton et al, 2013]. A collaborative group in the United Kingdom has also recently published the complete genome sequences of both another EEHV1A strain (Raman) and an EEHV1B strain (Emelia), including defining the physical ends of the virion DNA molecule. [Wilkie et al, 2013].
Similar analyses have also revealed two major chimeric sub-types of EEHV5 in Asian zoo elephants [Atkins et al, 2013; Denk et al, 2012]. So far four examples of EEHV5A and one of EEHV5B have been studied that differ by 10 to 20% in their gB-POL, TK-U49, gVPCR1 and UDG-gL-ORFO domains in a very similar fashion to the EEHV1A versus EEHV1B patterns. Even the very small segments of EEHV3 and EEHV7 evaluated so far from both wild and zoo African elephants show clear evidence for clustering of the multiple examples identified into two distinctive sub-groups each. The differences are recognizable at all five PCR loci available (U38/POL, U66/TERex3, U71-U72/gM, U73/OBP and U76/POR-U77/HEL), but are most pronounced (between 8 and 17% different at the nucleotide level) within U71-U72/gM and U73/OBP.

Other variable genomic features recognized so far include the absence of one copy of an anciently duplicated glycoprotein gene (ORF-Q) from EEHV2 compared to both EEHV1A and EEHV1B, and the deletion of a small vCXCL ligand-like gene (ORF-N) from all EEHV1B strains compared to all EEHV1A, EEHV2, EEHV5 and EEHV6 strains examined. Most noticeably, whereas EEHV1A and EEHV6 have diverged relatively uniformly from each other by about 16% all the way across their genomes and similarly EEHV2 and EEHV5A have done so by about 18%, both EEHV1B and EEHV5B evidently evolved much more recently as mosaic genomes formed by chimeric recombination events that incorporated several small segments acquired from additional related EEHV-type genomes (quite possibly derived from presumed mammoth EEHVs for example).

Therefore, current evidence indicates that the two most highly pathogenic Proboscivirus types, EEHV1A and EEHV1B, which evidently have been the cause of 90% of the fatal cases of acute systemic hemorrhagic disease seen in North America, Europe and Asia, together with EEHV4, EEHV5A and EEHV5B, are likely all natural endogenous viruses of Asian elephants. In contrast, EEHV2, EEHV3A, EEHV3B, EEHV6, EEHV7A and EEHV7B (but not EEHV1) have all been found within benign lung or skin nodules from healthy adult African elephants in Kenya and in North America. Together with five known species of elephant gamma herpesviruses (EGHVs) [Latimer et al, 2011], that fall into three very highly diverged branches (genus level at 40 to 60% different from one another), including three that have separate A and B versions (3.5% different) in African versus Asian elephants, a total of 12 distinct species and at least two sub-species of elephant herpesviruses have now been identified. The EGHVs are commonly found in oral and genital lesions and conjunctival swabs from healthy adult elephants, but none of have yet been associated with any significant disease conditions.


Seven Species of Elephant Endotheliotropic Herpesviruses (EEHVs) Represent a New Deltaherpesvirus Sub-Family.

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Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.
### New Suspected or Recently PCR Confirmed Lethal Cases

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>VLPs</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>Thailand</td>
<td>??? 2013</td>
<td>EEHV1A (S. Shripiboon)</td>
<td>D1</td>
</tr>
<tr>
<td>2x UK</td>
<td>Jul 2013</td>
<td>??</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>Jun 2013</td>
<td>??</td>
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<td>Singapore</td>
<td>Jan 2013</td>
<td>EEHV1 (M. Abraham)</td>
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<tr>
<td>Myanmar</td>
<td>2009-2012?</td>
<td>10x (plus)? (Zaw Oo/Gajah)</td>
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<td>Nepal</td>
<td>Nov 2012</td>
<td>EEHV1A (Suraj S, Dibesh K)</td>
<td>??</td>
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<tr>
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<td>Aug 2012</td>
<td>EEHV1A (C. Stremme, A.Z.)</td>
<td>2x C2</td>
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<td>UK</td>
<td>2011 EP#24</td>
<td>EEHV5A (Denk)</td>
<td>Vijay</td>
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<tr>
<td>2x Germany</td>
<td>2011 EP#23, 25</td>
<td>EEHV1A (S. Voight)</td>
<td>2x D1</td>
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<tr>
<td>Thailand</td>
<td>2011</td>
<td>EEHV4 (S. Shripiboon)</td>
<td></td>
</tr>
<tr>
<td>Thailand</td>
<td>2011?</td>
<td>EEHV1A (S. Shripiboon)</td>
<td>A2</td>
</tr>
<tr>
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<td>2011 IP#60</td>
<td>EEHV1A (A. Zachariah/S. Long)</td>
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<td>A2</td>
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<td>2011 IP#93</td>
<td>EEHV1B (A. Zachariah/S. Long)</td>
<td>B1</td>
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<tr>
<td>USA</td>
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<td>2009 IP#43</td>
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<td>D1</td>
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<tr>
<td>UK</td>
<td>2009 EP#22</td>
<td>EEHV1A (G. Wilkie)</td>
<td>Raman D1</td>
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<td>2x UK</td>
<td>2009 EP#20,21</td>
<td>EEHV1A</td>
<td>2x A2</td>
</tr>
</tbody>
</table>

Total Cases (North America): Deaths = 28 (21); Survivors = 7 (7)

Total Cases (Europe): Deaths = 26 (12); Survivors = 2

Cases (Asia) – anecdotal: Deaths = > 40 (17).
Acidophilic Cowdrey-A Nuclear Inclusion Body in Vascular Endothelial Cell In Asian Heart Tissue.
1. Over 60 cases of EEHV hemorrhagic disease in Western Zoos (80% lethal), and between 30-40 deaths in Asia. Extremely high levels of EEHV DNA in blood and necropsy tissue of viremic cases.

2. Eight different species/types of EEHV identified. EEHV1A, EEHV1B, EEHV4 and EEHV5 have all been lethal in Asians (Elephas maximus) but two chimeric versions of EEHV1A and EEHV1B predominate.

3. EEHV2, EEHV3, EEHV6, EEHV7 found in lung and skin nodules in Africans (Loxodonta africana). Only three known deaths.

4. Many monitored zoo Asians occasionally shed EEHV1A, EEHV1B or EEHV5 in trunk washes, and in some animals all three sequentially.

5. >36 strains of EEHV1A and 6x of EEHV1B identified. Many facilities have had cases of both EEHV1A and EEHV1B and multiple examples of the same strain, but strains found at different facilities always different.

6. Cannot yet be grown in cell culture—work with necropsy samples.
EVOLUTIONARY TREE OF MAMMALIAN HERPESVIRUSES (300 million years)

DNA POL (500-bp) (CODEHOPS)

NEW DELTAHERPESVIRUS = PROBOSCIVIRUS GENUS

7x EEHV species
5x EGHV species

α2

α1

δ

β3

β2

β1

γ2

γ1

HHV6A,6B,7

HCMV

KSHV

Elephant Gamma HVs

EBV

Proboscivirus (100 million y)

HSV1,2

VZV

Proboscivirus genus

Proboscivirus

Proboscivirus

Proboscivirus

Proboscivirus
DELTAHERPESVIRINAE

PROBOSCIVIRUS GENUS

GC-rich
63%

AT-rich
42%

No of Deaths/ Examples

Year of Discovery

EEHV3
EEHV4
EEHV2
EEHV5
EEHV1A
EEHV1B
EEHV6
EEHV7

NAP42 LA
*HANSA LA
*KIJANA LA
*NAP28 EM
*KUMARI EM
*NAP35 LA

(1/8)
(2/2)
(2/5)
(1/5)
(35/40)
(4/8)
(0/3)

2010
2007
2007
1999
2008
1999
2001
2009

* = lethal acute disease

# = 1B = chimeric genome

*HANSA LA
*NAP22 EM
*NAP28 EM
*KUMARI EM
*KIBA EM

(0/6)
(1/8)
(2/2)
(2/5)
(35/40)
(4/8)
(0/3)
PCR DNA Sequence Data Available

177-kb

EEHV1A(K)

EEHV1B(K-B, Ehlers)

EEHV1B(H)

EEHV6 (2x)

EEHV2 (2x)

EEHV5A

EEHV5B

EEHV3A,B

EEHV4

EEHV7A,B

60-kb

30-kb

50-kb

30-kb

66-kb

21-kb

22-kb

40-kb inversion

Unique-L

III - II - I

Core

90-kb

28-kb

Unique-R

MDBP

POL

RRA

TER

HEL

UDG

177-kb

PCR DNA Sequence Data Available
EEHVs = Deltaherpesviruses

Total EEHV1(Kimba) genome = 177,316-bp, 115 genes. Even the most conserved “core” genes of EEHV species differ by > 50% at the protein level from their nearest orthologues in other herpesviruses. Some retain only 25% protein identity to closest relatives the Roseoloviruses or show no viral homologues at all in BLAST searches.

EEHVs also encode 60 novel genes plus TK, RRB & OBP (and ori-Lyt) typical of alphas, but usually absent in Betas, and have a 40-kb inversion of the core gene block.

Based on intermediate position between Betas and Gammas in phylogenetic trees, plus the unusual overall gene content and organization, the EEHVs are sufficiently different from all other mammalian herpesvirus types to be classified as a distinct Deltaherpesvirus sub-family.
EEHV1A (Kimba)
177,236-bp, 115 genes

Common Core Genes

IE-like, 1300aa ORF-L
EEHV1A (Kimba) 177,236-bp

Captured/Pirated Cellular Genes

vGPCR1 vGPCR2 vGPCR3 vGPCR4 vGPCR5 vGPCR6 vGPCR7 vGPCR8

vOX2-2/3 Old vOX2-1 New

vGCNT1 Ori-Lyt

vCXCL inh?

6x CRE Pal 6x vgFam

vFUT9 =7TM

IE-like, 1300aa

Ori-Lyt
Protein

Alpha

Beta

Delta

Gamma

U48/gH Protein
U73/OBP Protein

Missing in Gammas and Betas except Roseolos

Alpha

Delta

Roseolo
U48.5/TK Protein

Missing in Betas and Roseolos

Delta

Gamma
EEHV1A vs EEHV1B Chimerism and Hypervariable Subtype Clusters

35 strains of 1A and 6 strains of 1B evaluated by multi-locus PCR sequencing. Overall DNA differs by > 4.3%.

Ancient chimeras but with over half = /< 1% different only.

Three 1A vs 1B-specific core domains (I,II,III) differ by 17% (gH/POL, 3.2-kb), 32% (gN/gO/gH/TK, 3.8-kb), 42% (UDG/gL, 6.5-kb) often = > difference between EEHV1A and EEHV6.

I, II, III are all linked, but other variable genes are scrambled (i.e. unlinked) especially within unique segment, including a multigene block at one end (IV, vFUT, vGPCR7/8, 6x vIgFam).

Additional hypervariability with 4-6 subtype clusters in at least 10 genes incl gH, vGPCR1, 4, 5, 8, vIgFam & vOX2-2/3.
World-wide EEHV1 strain subtype patterns

Figure 3:

vGPCR

<table>
<thead>
<tr>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
</tr>
<tr>
<td>A</td>
</tr>
</tbody>
</table>

- INDIA
- NORTH AMERICA
- EUROPE

gH-TK

<table>
<thead>
<tr>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
</tr>
<tr>
<td>A</td>
</tr>
</tbody>
</table>

- INDIA
- NORTH AMERICA
- EUROPE
EEHV1A/B Genome Hypervariability

Linked 1A/1B Chimeric Patterns
- vGCN1
- vGPCR2
- gB
- gN, gO
- gH, TK

Multiple Unlinked Subtype Clusters
- vGPCR5,6
- vOX2-2
- vOX2-3
- gH
- vGPCR1
- vGPRC1
- vFUT

20x 7TM

177-kb

Novel

Inv

Core Herpes Block

ORF-L(IE)
Pseudo-Species, Subtypes and Chimerism (Human herpesviruses)

HHV6A 30% 5% 30%
HHV6B

Linked diaspora

EBV-A 45% 15%
EBV-B

EBNA2
EBNA3A,B,C

KSHV-P 30%
KSHV-M
KSHV-N

K1/VIP 0.5%
A/C, B, D/E

A/C D
B, Q, R, N

PA/D/B 2% 2%
P A/D/B
M*=70%
N*=24%

K15/TMP

HCMV 20x hypervariable loci = ave 8x subtype clusters each but order completely scrambled by recombination = “swarm”
EEHV1A/B Subtype Divergence – Region III

143-kb

EEHV1A

HEL

ORF-M

1.6

1.0

ORF-O

gL

vCXCL

0.3

ORF-L

155-kb

IE-like

ORF-K

ORF-P

ORF-Q

S/T

Dupl

6.3-kb

EEHV1B

Absent in all EEHV1Bs, present in 1A,2,5,6,

EEHV2

23% 30% 38

29/31% 65%

37% 25%

Absent in EEHV2

Absent in all EEHV1Bs, present in 1A,2,5,6,
EEHV1 Subtype Divergence – Region IV

166-kb 175.5-kb

vGPCR7  vGPCR8

vIgF2.3  vIgF2.2  vIgF2.5  vIgF3

EEHV1A (R)

EEHV1A (K)

EEHV1B (E)

Absent in EEHV1B(E)

Absent in EEHV1A (R)

Absent in EEHV1A (K)

Deleted in K

Number of subtypes

2x 4x 5x 3x 2x 4x 2x 4x

42% 40% 35% 18% 25% 40% 15% 10% 30%
EEHV Disease Pathogenesis

Epidemiology zoo cases—all facilities different strains.
- No evidence for spread between zoos
Multiple examples of two calves with same strain.
- Most likely disease is primary infection
Sporadic shedding in Asian herdmate trunk washes.
  - Quiescent/latent infections are very common
Multiple sequential infections by EEHV1A, 1B and 5.
  - Give limited immunity against other types
Same disease/multiple strains in the wild in India / Asia.
  - EEHV1 present in Asians for >>100,000 years
Latency in lung and skin nodules of African elephants.
  - Multiple infections common, but very different types
Need to factor in chimeric EEHV1A, 1B, plus 5 and 4.
  - Order and early timing of infection critical?
Why are EEHV1A and EEHV1B so much more pathogenic?
ACKNOWLEDGEMENTS:

Funding Sources:

**National Elephant Herpesvirus Laboratory, Smithsonian Zoological Park, Washington DC:**

- **International Elephant Foundation**
- **Ringling. Barnum and Bailey Elephant Research Fund**
- **Morris Animal Research Fund**
- **Smithsonian Institute**

**Johns Hopkins University: School of Medicine, Baltimore, MD**

- **Viral Oncology Program:**

  - **NIH NIAID Research Grant R01 AI2457**
  - **International Elephant Foundation, Morris Animal Research Fund**
FINDING ELEPHANT HERPESVIRUSES
Virginia Riddle Pearson
Guest Researcher, Department of Molecular Biology, Princeton University
Honorary Research Associate, Department of Vertebrate Zoology,
The Academy of Natural Sciences of Philadelphia

EEHV2, EEHV3A, EEHV3B (a new subspecies), EEHV6, EEHV7A, EEHV7B (a new subspecies) and
EGHV1A, EGHV1B (a new species), EGHV2, EGHV4
in Tissue Biopsies and Saliva from African Elephants
in Kenya and America 2011-2012
in Botswana (August 26, 2013) and South Africa (? 2013)
HERPESVIRUSES

• Co-evolve with their hosts
• Cause primary disease, establish lifelong infection
• Species-specific, cross-species infection can be lethal

Calf of “Babylon”, Samburu, Kenya
credit Virginia Pearson, 2009

“Nisha” d.12.1.07, Dickerson Park Zoo
Nautilus “99”
courtesy Elliott Jacobson, DVM, PhD
University of Florida
College of Veterinary Medicine
Zimbabwe “Nautilus 99” in Florida

courtesy Daryl Heard, DVM, University of Florida College of Veterinary Medicine
Nodules exist on wild African elephants.

A surprising discovery by Virginia Pearson, July 2009

Credit: Virginia Pearson, Samburu, Kenya, 2009
NODULES

RNALater RNA Stabilization Reagent@www.qiagen.com
6 mm punch biopsy, iodine popules@www.midwestvet.net
“Enthusiasm” Virtues Family, Samburu. Kenya
credit Virginia Pearson 2011
KENYA 2011 SUMMARY
PCR/Gel Electrophoresis/DNA Sequencing

Skin Nodules and Lung Biopsy

EEHV2
EEHV3A
EEHV3B (new subspecies)
EEHV6
EEHV7A
EEHV7B (new subspecies)

EGHV1B (new species gammaherpesvirus).
Detection of Herpesviruses in Saliva
USA 2012

Non-invasive buccal swabs for first MHC gene characterization

Human herpesviruses detected in saliva: HCMV, HSV, VZV

Jason Holloway,
Six Flags Safari Park
SIX FLAGS 2012 SUMMARY
PCR/Gel Electrophoresis/DNA Sequencing

Saliva

EEHV2
EEHV3A
EEHV3B new subspecies
EEHV6

EGHV1A
EGHV1B new species of gammaherpesvirus
EGHV2
EGHV4

16” swabs@www.puritanmedproducts.com
RNAprotect Saliva Reagent, RNA protect Cell Reagent@www.qiagen.com
# COMBINED SUMMARY

January 28, 2013

<table>
<thead>
<tr>
<th>samples</th>
<th>EEHV</th>
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<td>KENYA Nodules</td>
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<td>KENYA Nodules</td>
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</tbody>
</table>

Promega PCR Master Mix[@www.promega.com](http://www.promega.com)
QIAquick Gel Extraction Kit[@qiagen.com](http://qiagen.com)
DNA Sequencing[@genewiz.com](http://genewiz.com)
Do hemorrhagic deaths occur in wild African elephants

Does co-infection with elephant gammaherpesviruses affect pathogenesis of this disease?”
KALWESI WATER HOLE
Chobe National Park, Botswana
Keep in sight!!
Immobilization dart
RNALater RNA Stabilization Reagent@www.qiagen.com
Before and after biopsy
Antidote
FIRST RESULTS

BOTSWANA
August 26, 2011
EEHV viral gene sequences:
U66TER, U77HEL, U71/gM, U73OBP

<table>
<thead>
<tr>
<th>EEHV2</th>
<th>EEHV3A/3B</th>
<th>EEHV6</th>
<th>EEHV7A/7B</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.a#1, male, 4yrs old with Four Trunk Nodules</td>
<td>L.a#1, male, 4yrs old with Four Trunk Nodules</td>
<td>L.a#2, female, 3yrs old with One Trunk Nodule</td>
<td>L.a#2, female, 3yrs old with One Trunk Nodule</td>
</tr>
<tr>
<td>L.a#2, female, 3yrs old with One Trunk Nodule</td>
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</tr>
</tbody>
</table>
ELEPHANTS ALIVE
Dr. Michelle Henley
GPS Collarings, APNR, South Africa
RNALater RNA Stabilization Reagent@www.qiagen.com
RNAProtect Cell Reagent@www.qiagen.com
16” swabs@Puritanmedproducts.com
Ear Lesion
Balule Reserve, South Africa
DNEasy Blood and Tissue Kit.@qiagen.com
GenTegra DNA tubes @ www.integenx.com
GENSOLVE@integenx.com
FTAcards@Qiagen.com
ACKNOWLEDGEMENTS

Dr. Lynn Enquist, Chairman, Department of Molecular Biology, Princeton University

Dr. Gary Hayward, Professor, Viral Oncology Program, The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine
Colleagues: KENYA and USA  2011-2013

Virginia R. Pearson¹,²*, Heidi Robbins¹, David Daballen³, Chris Leadismo³, Jerenimo Lepirei³, Gilbert Sabinga³, Lucy King³, Iain Douglas-Hamilton³, Francis Gakuya⁴, Domnic Mijele⁴, Mathews Mutinda⁴, Jeremiah Poghon⁴, Moses Otiende⁴, John Kariuki⁴, Isaac Lekolool⁴, William Rives⁵, Kenneth Keiffer⁵, Jason Holloway⁵, Nicholas Way⁶, Oliver A. Ryder⁷, Gary S Hayward⁸

¹Enquist Laboratory, Department of Molecular Biology, Princeton University, Princeton, NJ, USA;  ²Academy of Natural Sciences of Philadelphia, Philadelphia, PA, USA;  ³Save The Elephants, Nairobi, KENYA; ⁴Kenya Wildlife Service Veterinary and Capture Services Department, Nairobi, KENYA; ⁵Six Flags Wild Safari, Jackson, NJ, USA; ⁶Six Flags Discovery Kingdom, Vallejo, CA, USA; ⁷San Diego Zoo Institute for Conservation Research, Escondido, CA,USA; ⁸Viral Oncology Program, The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins School of Medicine, Baltimore, MD, USA⁸
Elephants Without Borders/Botswana: Dr. Michael Chase, Kelly Landen, Larry Patterson, DVM

Elephants Alive/Save The Elephants South Africa: Dr. Michelle Henley, Cobus Raath, DVM

WESSA South Africa: Chris Galliers

SANPARKS South Africa: Peter Buss, DVM

University of Pretoria Veterinary Genetics Laboratory, South Africa: Dr. Cindy Harper, Susan Miller

Princeton University, USA: Matthew Aardema

University of Washington, USA: Dr. Samuel Wasser
“What is man without the beasts? If all the beasts were gone, men would die from a great loneliness of spirit. For whatever happens to the beasts, soon happens to man. All things are connected.”

Chief Seattle,
Suquamish Tribe
Q and A

Photography by Virginia R. Pearson, 2013
Elephant Endotheliotropic Herpesvirus (EEHV): Where we are, where we’re going.

Lauren L. Howard, DVM, Dipl. ACZM
Associate Veterinarian
Houston Zoo, Inc.
The Houston Zoo and EEHV

EEHV History at Houston Zoo

• 1977: 6 year old Katu dies @ Houston
• 1988: 4 year old Beau Thai dies @ Canada
• 1991: 3 year old Pearl dies @ Chicago
• 1997: 11 year old Kiba dies @ Germany
• 2000: 7 year old Singgah dies @ Houston
• 2004: 13 year old Kimba dies @ Houston
• 2008: 2 year old Mac dies @ Houston
The Houston Zoo and EEHV

EEHV History at Houston Zoo

• 2009: Partnership between Baylor College of Medicine and the Houston Zoo
The Houston Zoo and EEHV

EEHV History at Houston Zoo

• Goals of HZI/BCM Partnership:
  – Establish local, rapid EEHV PCR testing
  – Better understand elephant immunity to EEHV
  – Better understand epidemiology of EEHV
  – EEHV treatments and possible vaccine development
The Houston Zoo and EEHV

EEHV: The Great Unknown

- Antiviral efficacy against EEHV?
- Immunity in elephants related to EEHV
- Case definition and epidemiology of EEHV
- Cultivation of the virus in the laboratory
9th Annual International EEHV Workshop

• Houston, Texas USA
• January 27-29, 2013
• 70 participants from 6 countries
• 15 scientific abstracts

For copy of proceedings and/or summary:
• lhoward@houstonzoo.org
EEHV Workshop 2013

N. American Asian Elephant Population

- 2011 ZooRisk Population analysis
- Current population not self sustaining
- Declining at 1.6% annually
- Will have a bottleneck in 15-30 years
EEHV and N. American Asian Elephant Collection

• Population Model by Lisa Faust Dec 2012:
  – Evaluated impact of EEHV on population
  – Assume elephants that died of EEHV actually lived

• Compared this result to 2011 ZooRisk analysis
Eliminating EEHV alone:

- Doubles population size in 100 years
- Improves growth rate to be stable (not decline)
Combination of eliminating EEHV and increasing breeding:

- 97% chance to reach target population size:
- Best scenario of all of them considered
EEHV in North America since 1978:

- 109 elephants in susceptible population
- 21 EEHV deaths
- 13 deaths due to non-EEHV causes

EEHV is the leading cause of death in Asian elephants born in North America since 1978.
EEHV in North America since 1978:

• 21 deaths associated with EEHV infection
• 9 survivors of illness associated with EEHV infection since 1978
  – 5 survivors in the past 4 years

• Overall: 70% fatality rate
EEHV Workshop 2013

EEHV in North America since **2009**:

- 5 EEHV survivors
- 1 EEHV-associated death

We are making headway on this disease!
Case Definition for EEHV

• N. American captive born Asian elephants
• between 0.5 and 15 years of age
• clinical signs associated with hemorrhagic disease
• confirmation of EEHV infection via PCR or histopathology.

Figure 1. (A-D) Pathologic changes of EEHV-associated disease found during field necropsy. (A) Asian elephant with cyanosis of the tongue attributed to EEHV disease; (B) Epicardial surface of the heart (apex view) from an Asian elephant showing severe extensive hemorrhage attributed to EEHV disease; (C) Ventricular endocardial surface of the heart from an Asian elephant showing multiple focal areas of ecchymotic hemorrhage attributed to EEHV disease; (D) Serosal membrane surface of the liver showing diffusely scattered petechial hemorrhage attributed to EEHV disease; (E,F) Photomicrograph of two capillary endothelial cells containing typical basophilic intranuclear viral inclusion bodies from necropsy liver tissue. Hematoxylin and eosin stain, bar = 25 µm.
Case Definition for EEHV

• clinic signs associated with hemorrhagic disease
  – Which signs, how severe

• confirmation of EEHV infection via PCR or histopathology.
  – What level of viremia is significant?
EEHV Workshop 2013

Multi-year EEHV Epidemiology Study
Dr. Ramiro Isaza, University of Florida

20 Institutions both w/ and w/o EEHV surveyed

• No apparent association between illness or death from EEHV in Asian elephants and exposure to African elephants.
EEHV Workshop 2013

Dr. Gary Hayward/Johns Hopkins

EEHV1A complete genome compiled

- New virus family: Deltaviruses?

11 EEHV Sub-Types and Distribution

- Asian Elephants:
  - EEHV 1, 4, and 5
- African Elephants:
  - EEHV 2, 3, 6 and 7
EEHV and African Elephants

Baylor College of Medicine evaluated trunk secretions in healthy African elephants

EEHV shedding in 10 African elephants:

- 4/10: EEHV 3/4
- 4/10: EEHV 6
- 1/10: EEHV 1 *
  - *housed previously with Asian elephants
EEHV and African Elephants
Maryland Zoo / Dr. Bronson

• EEHV-3 associated illness in African calf
• Use of whole blood viral loads as measured by real time PCR to guide therapy
• Kudos to Veterinary and Elephant Team!
Immunity and Elephants

Dr. Carolyn Cray (U Miami) evaluated acute phase proteins in blood of elephants

- Serum amyloid levels 10X higher in EEHV viremic elephants
- Prognostic value
- Elephant immunity
Immunity and Elephants

Baylor College of Medicine looked at passive transfer of antibodies in Asian elephants

• Majority of maternal-fetal antibody transfer occurred through the placenta, rather than through colostrum.
EEHV Workshop 2013

Immunity and Elephants
Dr. Byron Martina (Erasmus Medical Center)

• Developing a gB based ELISA for evaluation of EEHV antibodies in elephants
• Have run samples from Europe and the US through the ELISA, results under evaluation
Clinical Management of EEHV

Twycross Zoo/ Dr. Sharon Redrobe

• EEHV-5 associated illness and death in an Asian elephant
• Use of methadone improved attitude
• First time pericardiocentesis was performed
Changes at National Elephant Herpesvirus Laboratory (NEHL)

• No longer performing free EEHV testing
• Facilities needing testing may
  – Join the NEHL Consortium
  – Pay individually per test

For more information: Erin Latimer at NEHL (202) 633 4252 or latimer@si.edu
EEHV Workshop 2013

NEHL Consortium (different levels available)

Advantages of Membership

• Unlimited diagnostic testing of elephants
• Routine calf screening****

For more information: Erin Latimer at NEHL (202) 633 4252 or latimer@si.edu
Advantages of Membership

• Testing & Subtyping for all EEHV
• Meeting AZA elephant accreditation standard for Conservation and research activities
• Media support on EEHV issues, if needed
EEHV Workshop 2013

www.eehvinfo.com
Future Areas of Focus

- Establishing funding to support research
- Further refinement of epidemiology of EEHV
- Better understanding of elephant immune response related to EEHV
  - EEHV Serology test on the horizon
EEHV Workshop 2013

Future Areas of Focus

• Continued attempts to culture virus
• Ongoing work to confirm antiviral efficacy
• Laying groundwork for vaccine development
EEHV Workshop 2013

Acknowledgements
Co-Author: Dr. Paul Ling, Baylor College of Medicine

Questions?

HZI Elephant Team

HZI Veterinary Department

Rick Barongi, HZI Director
Sharon Joseph, HZI VP Animal Operations
The Lonely Rhino: Analyzing Anthropomorphism Toward Solitary Animals

By: Selenia Murillo
Introduction
Mission

To inspire conservation leadership by connecting people with wildlife and nature

Chicago Zoological Society
Top 5 Favorite Animals

1. Lion and Lioness
2. Giraffes
3. Dolphins
4. Gorillas
5. Brown Bear
6. Elephant
7. Rhinoceros
I prefer to see animals in group settings.
I prefer to see animals in group settings.
Are we effectively fulfilling our mission?
Methods
2. Using the scale below, please indicate how much you agree or disagree with the following statements about the black rhino.

<table>
<thead>
<tr>
<th>Strongly disagree</th>
<th>2</th>
<th>Moderately disagree</th>
<th>Slightly disagree</th>
<th>Neither agree nor disagree</th>
<th>Slightly agree</th>
<th>Moderately agree</th>
<th>Strongly agree</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>a. The black rhino appears lonely.</td>
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<td>b. The black rhino appears healthy and well-fed.</td>
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<td>c. The black rhino seems stressed by the presence of visitors.</td>
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<tr>
<td>d. The black rhino has adequate space.</td>
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<td>e. The black rhino’s behavior differs within a zoo setting.</td>
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<tr>
<td>f. The black rhino has enough toys and interactive features in its exhibit.</td>
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<td>g. The black rhino doesn’t need a companion.</td>
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<td>h. The black rhino appears sickly and malnourished.</td>
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<tr>
<td>i. The black rhino’s environment lacks engaging and enriching stimuli.</td>
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<td>j. The black rhino’s behaviors are similar to those of wild rhinos</td>
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<td>k. The black rhino needs more room.</td>
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<tr>
<td>l. The black rhino was unaffected by the presence of visitors.</td>
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<td>m. Select the number 5 for this item.</td>
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</table>

3. Based on the behaviors you observed, how would you describe:
   a) the black rhino’s mood or emotional state __________________________________________

   b) your mood or emotional state __________________________________________

4. Which best describes the natural social structure of black rhinos?
   □ Solitary □ Pair Bond □ Small group □ Herd □ Fission-fusion community □ Other _________________
Pachyderm House
Results
Visitor Demographics

- **Membership**: 54% Members (n=191)
- **Gender**: 56% Female (n=200)
- **Age**: 39% within 30-39 range, 27% within 40-49 range (n=171)
- **Ethnicity**: 83% Caucasian (n=171)

86% completed most or all of the survey
What % of visitors think that black rhinoceroses are social?

n=181
Black Rhino Activity

16% Inactive
- Not alert
- Alert

84% Active
- Moving around
- Eating
- Interacting
- Pacing
- Manipulating objects

n=338
## Animal Concern: Solitude

### The black rhino appears lonely.

<table>
<thead>
<tr>
<th>Agree</th>
<th>Neutral</th>
<th>Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>47%</td>
<td>32%</td>
<td>22%</td>
</tr>
</tbody>
</table>

### The black rhino doesn’t need a companion.

<table>
<thead>
<tr>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>63%</td>
<td>27%</td>
<td>10%</td>
</tr>
</tbody>
</table>

n=146
Animal Concern: Health

The black rhino appears healthy and well-fed.
- 87% Agree
- 6% Neutral
- 6% Disagree

The black rhino appears sickly and malnourished.
- 87% Disagree
- 5% Neutral
- 7% Agree

n=151
### Animal Concern: Stress

<table>
<thead>
<tr>
<th>The black rhino seems stressed by the presence of visitors.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Agree</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The black rhino seems unaffected by the presence of guests.</th>
</tr>
</thead>
<tbody>
<tr>
<td>11% Disagree</td>
</tr>
</tbody>
</table>

n=152
Animal Concern: Space

The black rhino has adequate space.

- 44% Agree
- 15% Neutral
- 41% Disagree

The black rhino needs more room.

- 18% Disagree
- 19% Neutral
- 63% Agree

n=150
Animal Concern: Behavior

<table>
<thead>
<tr>
<th>Statement</th>
<th>Agree</th>
<th>Neutral</th>
<th>Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>The black rhino’s behavior differs within a zoo setting.</td>
<td>29%</td>
<td>55%</td>
<td>16%</td>
</tr>
<tr>
<td>The black rhino’s behaviors are similar to those of wild rhinos.</td>
<td>26%</td>
<td>62%</td>
<td>12%</td>
</tr>
</tbody>
</table>

n=138
### Animal Concern: Enrichment

**The black rhino has enough toys and interactive features in its exhibit.**

- **37%** Agree
- **20%** Neutral
- **43%** Disagree

**The black rhino’s environment lacks engaging and enriching stimuli.**

- **28%** Disagree
- **17%** Neutral
- **55%** Agree

\( n = 147 \)
Emotional Responses

Rhino’s Mood or Emotional State

20% Negative Descriptors

- 54% Bored
- 32% Lonely
- 19% Sad

n=185

Visitor’s Mood or Emotional State

3% Negative Descriptors

- Sad for the rhino
- Concerned about space
- Content, but worried he’s sad

n=179
<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all</th>
<th>Somewhat</th>
<th>Very much so</th>
</tr>
</thead>
<tbody>
<tr>
<td>How much did visiting the black rhinos influence your decision to come to the zoo today?</td>
<td>73%</td>
<td>17%</td>
<td>10%</td>
</tr>
<tr>
<td>Did you have an up-close encounter with a black rhino today?</td>
<td>30%</td>
<td>23%</td>
<td>48%</td>
</tr>
<tr>
<td>Did you make eye contact with a black rhino today?</td>
<td>39%</td>
<td>16%</td>
<td>46%</td>
</tr>
</tbody>
</table>
Visitor Comments

33% noted animal concerns
- Improve exhibits
  - More indoor space
  - Naturalistic
  - Increase outdoor access
  - Provide hiding places
- More animals

29% mentioned elephants

n=49
The black rhino doesn’t need a companion.

\[ r_s(145) = -0.345, \ p = 0.000^{**} \]

The black rhino appears lonely.

\[ r_s(144) = 0.186, \ p = 0.026^* \]

The black rhino seems stressed by the presence of visitors.

\[ r_s(145) = 0.166, \ p = 0.046^* \]

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level
Behavior

The black rhino appears lonely.
\[ r(194) = -0.198, \ p = 0.006^{**} \]

The black rhino appears healthy and well-fed.
\[ r(200) = 0.159, \ p = 0.024^* \]

The black rhino is unaffected by the presence of visitors.
\[ r(196) = -0.164, \ p = 0.022^* \]

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level
Up-close Encounter

The black rhino appears healthy and well-fed.

\[ r(126) = 0.232, \ p = 0.009^{**} \]

The black rhino’s environment lacks engaging and enriching stimuli.

\[ r(125) = -0.185, \ p = 0.039^{*} \]

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level
Eye Contact

The black rhino appears healthy and well-fed.
\[ r(125) = 0.237, p = 0.008^{**} \]

The black rhino has enough toys and interactive features in its exhibit.
\[ r(122) = 0.272, p = 0.002^{**} \]

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level
Eye Contact

The black rhino’s environment lacks engaging and enriching stimuli.

\[ r(124) = -0.259, p = 0.004** \]

The black rhino doesn’t need a companion.

\[ r(123) = 0.180, p = 0.046* \]

The black rhino has adequate space.

\[ r(122) = 0.179, p = 0.049* \]

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level
Conclusion
Summary

• Majority of guests are unaware of the black rhino’s solitary social structure
  - More inclined to think black rhino is lonely and stressed
Summary

- Active black rhinos are seen as less lonely, less stressed, and healthier
- Up-close encounters and eye contact improve perception of the black rhino’s welfare from the visitor’s perspective
Next steps for black rhino conservation

1. Accomplish mission
2. Increase education
3. Conduct more research
Acknowledgements

Animal Programs

• Susan Hoss
• Scott Katzberger
• Rick Lichner
• George Morgan

Conservation Education and Training

• Debra Kutska
• Jerry Luebke
• Jennifer Matiasek
Questions?

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PRESENTATIONS
SESSION VII

EX SITU BENEFITS AND SUPPORT OF IN SITU CONSERVATION
The Bigger Picture: How captive elephant facilities can benefit wild elephant populations

Sean Hensman

Manager, Adventures with Elephants

Bela Bela, Limpopo, South Africa.

We got our elephants in 1988, not to have as pets or to do Safaris, but to release onto a once commercial cropping farm which dad had converted into a Game Reserve so that we, as his children, could grow up learning about a range of animals in a wild environment. Dad enjoyed the African bush and this was his attempt to bring the Zambezi valley a little closer to home for all of us. We got our first elephants from culling operations in Zambezi and they arrived at 45 inches tall. We realized that they were too small to release as we had originally intended, so we put them in an enclosure and looked after them. This is where we learned that there was so much more to elephants than we had originally expected. They were eager to interact and within a few weeks they were following a handler about the farm. As time went on we got more and more orphans and problem elephants and as such landed up with a herd of 12 elephants on the farm.

If you have pets at home or look after animals you will understand how expensive they are, so we made them earn their keep by doing farm chores such as rounding up cattle, checking fences and conducting wildlife patrols.

As time went on we had more and more visitors coming out to visit the elephants which was one of our interests, and since the expenses were covered by the farm and dad liked people learning about the elephants in this unique manner, we allowed people to interact with them for free. Though the elephants weren’t originally planned to be our pets, we enjoyed spending time with our elephants, as one would with their pet dog or horse, great symbiotic relationships happened between us and the elephants, we landed up doing some incredible things with them.

However, Dad realized that he could reach a wider audience and teach a wider range of people about the elephants if he got into tourism, so our first herd was sent up to Victoria Falls in 1993 and began doing Elephant Safaris. After that we got another 12 elephants which we started to train for anti-poaching in the Zambezi Valley where 33,000 rhino were lost in a 20-year period. Dad’s plan was to assist anti-poaching teams with their mobility issues, as the great thing with the elephant is that they can go anywhere, were built for all the African conditions, never need to be refuelled or retreaded and can follow a human scent. Incidentally we successfully used them to track down a robber who had broken into our next door neighbour’s house in Zimbabwe when the police dogs couldn’t.

Unfortunately we had to leave Zimbabwe before we could get involved in anti-poaching and moved to South Africa in 2002. The elephants provided us a lifeline and since then we have carried on with public elephant activities.

Today we bring in problem elephants that are going to be destroyed and give them a second chance by training them for public education activities, telling people about the elephant in general as well
as the plight facing wild populations and conservationists in an ever changing Africa. The first elephant we tamed in South Africa was Tembo, a 32-year old 6 ton bull. He caused over R1 million worth of damage to a game lodge in 6 months; he broke into buildings, turned over vehicles, killed buffalo and even rhino. One can imagine that he was not a very popular elephant, and he was going to be destroyed. Today he is educating people at Elephant Whispers in Hazyview, near the Kruger National Park.

We are not a government funded operation and earn a living from our elephants. The elephants were always seen as part of our family and we get immense joy from them, in turn we looked after them and today it is a privilege working with them and introducing people to them while highlighting the difficulties they face in an uncertain world. We also conduct a range of interesting research with our elephants in a quest to learn more about them, as they have given us so much that we are always trying to find ways to give back to their continued conservation and to come up with conservation solutions for our elephant’s wild cousins.

Having captive elephants is a hotly contested issue, and there are people who will agree with what we do and people who will disagree with what we do. I try understand both sides of the argument, however they are vast and complex due to issues such as emotion, differing perspectives, different goals, egos, and more. Many people do not see, or do not want to see, the value of having elephants in captivity and I understand why they feel like this. Though we have our own captive elephants we too would like to see all elephants roaming free, but the reality is that the world has changed and today WE NO LONGER LIVE IN AN IDEAL WORLD. If we did we wouldn’t be wearing fancy clothes or depleting the ozone by burning jet fuel to come to America to talk at an international conference about the problems surrounding the conservation of African and Asian animals. So we feel that a compromise between the two extremes is acceptable, especially if our captive elephants can assist wild elephants.

One thing is VERY clear; as the human population grows and develops its only going to get worse for wildlife as ultimately, the root causes of all the issues in the world today are due to us humans in one way or the other. The old world our fathers experienced is long gone, the reality remains that many of the remaining elephants in the wild are being forced into small and diminishing, enclosed reserves where their own safety or survival cannot be guaranteed and we have to find a variety of solutions to ensure their future fast! If some people’s predictions are right there will probably be no elephants left in the next few years in many countries that currently have wild elephant populations – and it is also happening to the rhino at a rapid pace.

There are those that believe in wildlife remaining wild and those who see potential in working with wild animals in captivity. If we don’t find ways of working together or even possibly agreeing to disagree, we will waste valuable time, money and energy arguing with each other instead of finding a common understanding and finding solutions for the conservation of wildlife in a rapidly changing world, where animals come second to commerce and human needs or greed. Most of us ironically benefit from elephants in one way or another (through donations for research, entry fees to zoos or facilities such as ours, donations to save them, entry fees to national Parks to see them etc.) , and ultimately we all have the same goal: that our grandchildren’s children can experience elephants in the wild, but we have different ways of reaching that single goal.
I’m not saying that I have the answers, but I am saying that the potential for captive facilities to assist wild elephant populations is incredible if it is done in a correct and in an ethical manner. Not everyone is able to live with and observe elephants in the wild, and not everyone is able to work with them in captivity, so it is our responsibility to do our level best to make sure that people appreciate elephants and nature! We should all be working together toward a common goal as WE all WANT ELEPHANTS IN THE FUTURE!!

We are about making connections, connections between humans and animals. These connections are created through education and hands-on experience which results in understanding. Through these interactions, elephants become emotionally valuable to humans, through association and understanding.

Education is paramount and we all push to enlighten the public about elephants in one way or another, researchers do work to find out more about elephants and then publish their work for the world to see. Captive facilities allow the public access to easily observe or interact with elephants. In addition there are other outlets for education, there is the radio, internet and television and we all take advantage of these.

Our main focus is to allow people to simply let go and enjoy the elephants as we do, and see more in them than people give them credit. We do a variety of different things with the elephants to show people their values and how incredible they really are. Our aim is to educate the public about elephants, their biology, ecology and conservation. We feel that guests are able to experience a captive elephant in more ways than a person might normally be able to experience an elephant from watching it in the wild or even on television in their home. Our hope is that the next time a person watches an elephant feeding in Kruger they’ll think about how soft the elephants tongue is, or what the environmental impact is of the elephant in that area.

We invite them into the elephant’s world, where our visitors can learn in the most primal of fashions, through experience. We allow guests to see, smell, hear, touch and yes, even lick the elephant if they want to! All this is done so that people can get to understand the elephant better. In addition our guests can experience the personalities, intelligence and wonder of each elephant, whilst both they and the elephant are engaging with one another. We reach roughly 12,000 people per year at our facility alone, in Southern Africa all the elephant facilities combined reach roughly 300,000 people annually. I know that these numbers pale in comparison to the many millions of people who visit the Serengeti, Okavango or even Kruger National Parks. The difference is that our focus is solely about the elephants and not on what could be around the next corner. In addition, the visitors paying for these experiences are the people in the position and perhaps best-suited to affect change as they are the “have’s” in society who are able to travel the world, take holidays and use their money and influence to support a good cause - which is exactly why education is the key.

Education has to be fun in order to keep a child’s, as well as an adult’s attention. Swims – put people right into the middle of a herd of elephant, humans in this scenario are vulnerable but the elephants play gently around them. People leave amazed at how the elephants have so much fun diving and playing in the water as a family but also how aware they are of the people riding them. When we get an elephant to spray water over a kid they will remember forever just how much water an elephant can hold in its trunk and needs daily in order to survive!
What about the “have not’s” in society, the people who can’t afford life’s luxuries of spending a holiday in the wild observing wild animals at leisure, or even worse what about the people who live with wildlife surrounding them which affects their daily lives. It’s really these people who need to understand the value of wildlife and what they have in their back yard. Through our Rural Education Programs, we invite rural communities to the elephants and show them that their problems may actually be great opportunities. We try to reach out to these communities at every opportunity to show them an elephant as well as what elephants are, so that they might make decisions in their futures about the elephants in their back yard.

It is incredibly important that these rural people lose their fears and inhibitions regarding the elephants and no longer see them as problem animals or animals to be afraid of, but they see them as intelligent, sentient and gregarious beings that have been caught up in humans drive to take over the world.

To support education programs, we try our best to promote the elephants at every opportunity, to constantly put elephants into everyone’s lives. One of the best places to do this is on television. Over the years we have been involved in many documentaries where stories about elephants had to be recreated for very specific scenarios recreating behaviours which would have been impossible to capture on film with a wild elephant. We have done work for most of the big brand production houses and re-enacted everything from elephant trampling’s which highlight HEC and dramatic elephant releases all the way through filming elephants doing what they do naturally, swimming, breaking trees and foraging in the bush. These incredible images of elephants are able to be captured with our elephants and beamed to television sets around the world expanding knowledge and hearts in your own home. We have done commercials for worthy causes and about incredible people involved with elephants, pushing their stories and hopefully inspiring the public to look deeper at elephant conservation.

Captive Elephants can physically show all the wonders of why elephants should be protected, their intelligence, capabilities, nature etc. We open people’s eyes and minds to elephants by allowing them to enter the elephant’s world and by allowing them to experience the elephant in so many amazing ways that we take for granted every day. We forget how lucky we are to work with elephants, and that our guests often comment that this is a “once in a lifetime experience”. This is the key to opening the door on the bigger picture facing elephants in an ever changing Africa.

Yes, these elephants didn’t choose to be here interacting with people, but neither did the elephants in the Kruger National Park choose to be in that park, humanity chose for them at some stage in the past. We simply feel that it’s our responsibility to ensure that our interactions and decisions are not to the detriment of the elephants, but rather to ensure that we do the best that we can for the elephant and highlight their positive and negative attributes along with their problems.

In an attempt to understand elephants in more detail, we have conducted a lot of research over the years. I believe Daphne Sheldrick hits the nail on the head when she said, “I have grown up watching wild elephants and I have experienced living and working with captive elephants and as such I am in an ideal position to know so much about them”. And looking around the room I think you will agree that we are all doing related work however, each is different because of restrictions imposed on them. We have all learnt much from captive elephants. A simple search through Google Scholar divulges a plethora of research on captive elephants, enlightening field biologists across the globe.
Topics include: behaviour, tool use, disease control and management, cognitive behaviour, reproductive cyclicity, chemical signalling, vocalization studies, seismic communication and many more...

Many incredible and well-respected researchers started their careers looking at captive elephants. Katy Payne’s career was launched by observing elephant behaviour in Oregon Zoo, and Joyce Poole learnt from a paper on captive Asian elephants that what she was witnessing in wild African elephant bulls was not “Green Penis syndrome” as she initially called it, but the state of musth. Ms Sheldrick too, working with elephant orphans on her game farm in Kenya, developed a milk formula she has since shared with elephant keepers worldwide.

Our main focus is education of the public but we do push research at every opportunity and are in the process of setting up a research centre at our facility. After all knowledge leads to greater understanding, which in turn allows us to make educated and informed decisions.

Our Research has developed over the years but started the day we got Jumbo and Miss Ellie in 1988. The first thing that we learned was how willing to interact with us the elephant were and how quickly they forgot and forgave the atrocities of their past. The other thing was how incredibly intelligent the elephants are and how quickly they picked up on things.

I think our facility bridges the gap between a truly captive environment and elusive wild elephants. The availability of land and free range feeding time allows researchers to observe elephant behaviour at close proximity and safely, while we are able to influence the elephant to obtain data. In the wild, one can obtain observational data. In our environment one can get more in depth observational data as well as experimental data.

One of our primary research goals is to try to find solutions for human elephant conflict (HEC). One of our most memorable research quests was to research chillies and how they might help alleviate HEC in Africa. It was a few fun filled months of dodging Kat Von Gerhard as when we saw her we knew that we would be smoked out of our office and would spend the rest of our day smelling like a bush fire with a twist! We watched as the elephants figured out how to avoid the chillies so we could then make quick changes for better results.

As we all know HEC threatens food security of rural farmers in Africa as well as India and protection of the elephants depends on support of rural populations who aren’t too keen on helping when an elephant eats their subsistence crop. So measures are being sought to mitigate HEC. Chilli is a potentially cost-efficient and effective way for a rural farmer to mitigate against HEC (Hanks, J. 2006). However, there is much debate among the elephant community as to effectiveness of chilli fences and chilli briquettes! So Katharina took 8 elephants to a 10m x 10m plot filled with 50 oranges. This plot was “protected” by varying strengths of capsicum on chilli grease fences and burning of chilli briquettes. We found that captive elephants reacted more strongly to smoke of chilli briquettes (concentration: 400gr chilli) than to chilli grease and that food-driven individuals were more risk tolerant. We discovered that wind direction and strength was important – wind had to blow directly on to elephants from nearby (<5m) for them to react to smoke. A strong wind caused the smoke to blow parallel to ground which was easy for the elephants to step over. We watched as the elephants always approached the field upwind, stepping over smoke, clearing nasal epithelium (snorting upon exhalation, trunk flicking and trunk sucking) and dust-bathing in response to chilli.
This study was then applied in Zambia. The fields with chilli experienced no serious raids, and much fewer elephant visits. Elephants would approach chilli grease fence (sisal string with chilli grease) and follow the fence, looking for a way in and then they raided a neighbouring unprotected field. The rural farmers were first doubtful, then supportive after witnessing results.

Our other research activities include:

- Can elephants detect landmines and learn to avoid them? Their sense of smell is said to be 14 times better than that of a good dog. Today we are working with the USARO and will be publishing our results in a few months time,
- Disease detection – Hoof and mouth, EEHV
- Elephant vocalizations
- Additional HEC mitigation techniques
- Nutrition
- Contraception
- Management of elephants in small game reserves

With the above in mind, I firmly believe that our elephants, as well as the elephants at other facilities around the world, are a vital link between the remaining wild populations and modern man. These tamed elephants are ambassadors for their wild cousins by highlighting the difficulties facing wildlife and conservationists in an ever overpopulated, changing and modernizing Africa and Asia. I believe that our facilities highlight tomorrow’s issues as well as today’s, and although irreversible decisions were made in the past, today we can do our best to help shape the future. The connections made between elephant and man can create understanding and in turn can inspire humanity to get out there and do something for wildlife conservation.
The Bigger Picture

How captive elephants facilities can benefit wild elephant populations.

Sean Hensman (Zebula Logo)
Adventures With Elephants, South Africa
Highlight the reality - Economics of conservation.

- Our 6 elephants were problem elephant.
- Today they are ambassadors for conservation
- We learn from them daily
Highlight the Economics of Conservation

- Lose a farm and eventually the elephant or make some tough decisions.
- What to do with ‘Problem’ elephants.
- No such thing as a free lunch, esp. in today's world.
Methods - our approach

- We were always hands on with all our pet animals and found that this is how people learnt to love animals and so people visited us, but more importantly we learnt about the animals.
- Realised that the elephant were eager to please, and saw potential in them as we learnt more about them; as well as from observing wild elephant.
- We took in problem elephant and gave them a second chance to do some good
WE CALL IT A "FENCE"!
KEEPS OUT THE GIRAFFES
AND THE LIONS AND THE
ELEPHANTS AND THE . . .

8000 BC

HUMAN POPULATION
DEMANDS

1800

WE CALL IT A "WILDLIFE
PRESERVE"! KEEPS IN THE
GIRAFFES AND THE LIONS AND
THE ELEPHANTS AND . . .

AD 1500
Connections
Education

- Hands on Elephant focused experience
- Numbers of people who visit captive facilities – they are an essential part of conservation.
- Rural populations
- The future
Education - Hands-on Experience
Education - Hands-on Experience
Education - Hands-on Experience
Fun - Swims
Incredibly special - weddings
Rural Children – Education Programs
Promotion

- Films and Documentaries (Taking the elephant message into homes around the world)
Connections – ensuring this result.
Research - Related Work

- **Captive facilities**
  - Zoos, Safari Operators, Elephant Parks.

- **Natural facilities**
  - Dame Daphne Sheldrick
  - National Parks,
  - Researchers.
  - Cynthia Moss & J Poole
  - Dame Daphne Sheldrick
Kenya's First Lady Margaret Kenyatta Fosters an Elephant.
Research and Future Potential

- How it started
- Observation & experience led to research
- Listening & marvelling led to research
HEC – Chilli Smoke

Katharina von Dürckheim
Department of Conservation Ecology & Entomology, Stellenbosch University
e-mail: kat@sun.ac.za
Future Work

- HEC Solutions
- Assisting elephants in small game reserves
- EEHV
- Contraception
Research & elephants future potential

- AWE actively pursue research in order to better understand elephants.
- Elephant specific
  - Stress – Isotopic research (Jacqui Condron)
  - Contraception – GNRH (Henk Bertsinger)
  - Communication – Origin of vocalization (Angela Stowarth)
  - HEC resolution – chillies (Katharina von Gerhardt)
Abilities – Bio Detection

Jessica Brown, Ph.D.
Postdoctoral Researcher, US Army Research Office
e-mail: jessicasbrown01@gmail.com
Origin of Vocalisation

Citation: Stoeger AS et al. (2012) PLoS ONE 7(11): e48907. doi:10.1371/journal.pone.0048907
In the end we will only conserve what we love, we will love only what we understand, we will understand only what we are taught.” (and Experience) Baba Dioum, Senegal.
Questions ?
Contributions to Science and Conservation by Elephant Managers and Captive Elephants

Heidi S. Riddle

Riddle’s Elephant and Wildlife Sanctuary
Who are ELEPHANT MANAGERS?
Elephant Managers Association

* Established in 1988 to promote elephant management

* Currently an international membership of about 500 individuals and institutions

* Annual conference

* Recognized as the most experienced group of elephant professionals
Indonesian Mahout Communication Forum

*Established in 2006 in Sumatra, Indonesia

*Members are mahouts from government camps and private facilities

*Annual mahout workshop

*Participate in activities with wild elephants such as conflict mitigation, radio collaring
Elephant Managers and Science
Ear Liquid of Elephant Origin
What is its function?

• Cleanser — doubtful — few phenols
• Lubricant — probable — higher alcohols
• Chemo-communicative — suggested by
  1. ear checks by other elephants
  2. compounds of similar nature
• Needs further study — anatomical, behavioral, and chemical
MODA MUSTH

- An elephant manager’s observation of honey odor in a young male Asian elephant

- Hindu Poetry: “allusions of bees coming and gathering sweetness from the temples of Musth elephants”
Moda Musth
Biological Convergence

Secretions from young males smell like honey

Same compounds (leaf alcohol) give honey their odor and are honeybee pheromones

Changing chemical scenario from Moda to Adult Musth

May serve to facilitate male-male interactions
Elephant Managers and Medical Procedures
Elephant Managers and Diagnostics
Trunk Wash
Elephant Managers and Treatment
Mouth Check
Mouth Block for Oral Dosing
Rectal Administration
Conservation Response Unit
Seblat - Bengkulu
1st Sumatra Mahout Workshop

November 2006
Training Module during Mahout Workshop
Way Kambas National Park

Conflict mitigation by elephant patrol teams
Law Enforcement
Prevention of Wildlife Crime
Habitat Restoration
Elephant Managers and Collaboration
Donation elephant care tools
THANK YOU!
Contributions of the Ringling Bros. Center for Elephant Conservation in Sri Lanka to Wildlife Research and Management

Dennis Schmitt\textsuperscript{1,2}, S. Wijeyamohan\textsuperscript{1,3}, Wendy Kiso\textsuperscript{2} and Charles Santiapillai\textsuperscript{1,3}

1 The William H. Darr School of Agriculture, Missouri State University, Springfield, Missouri, USA

2 Ringling Bros. Center for Elephant Conservation, Polk City, Florida, USA

3 Center for the Study of Asian Elephant at Rajarata University of Sri Lanka at Mihintale, Sri Lanka

“….In the final assessment it is understanding rather than sentimentality that will do most for the conservation of creatures other than ourselves”
Mrs. Pat Hall (1933) British Museum of Natural History.

Abstract

In order to enhance the management of wildlife in general and the elephant in particular in Sri Lanka, the Ringling Bros. Center for Elephant Conservation\textsuperscript{®} (Ringling Bros. CEC) in Polk City, Florida, USA hosted four Sri Lankan graduate students for one year of training and research, utilizing elephants at the Center, as part of their postgraduate degree program with the University of Peradeniya, Sri Lanka. Three of them have submitted their theses for review, while the fourth student’s research was upgraded to PhD. Ringling Bros. CEC subsequently built a bullpen for the Temple of the Tooth in Kandy to manage bulls in musth without them being chained. The partnerships with Sri Lankan universities, the Department of Wildlife Conservation (DWC), and Ringling Bros. CEC led to the surveys of human-elephant conflict in 2008, 2009 and 2011. Ringling Bros. CEC has also established the Ringling Bros. Center for the Study of Asian Elephant at Rajarata University in Mihintale, Sri Lanka and provided funds for student projects. In addition, it has provided resources to conduct a course on Wildlife Conservation & Management at Rajarata University. At the Elephant Transit Home (ETH) in Uda Walawe National Park, Ringling Bros. CEC has been assisting the DWC in monitoring the growth of the orphaned elephant calves by weighing them at monthly intervals for over three years. The data from weighing these calves has become a unique resource. In addition, the program is also assessing the changes in the body condition of wild elephants at monthly intervals. An accurate method to estimate the length and/or height of elephants and other wildlife using laser beams has been introduced. In addition, the decibel levels of the firecrackers that villagers use to ward off wild elephants were tested and found to be very high indeed. Frequent bursting of such firecrackers in close proximity to elephants may impair their hearing. An innovative method to monitor wild elephant movement was initiated using standing sedation during which a home-made GPS/GSM collar was affixed to the bull. The elephant’s movement was then tracked on line once every minute for a month. This technology was mainly developed to monitor marauding elephants with the aim of mitigating the human-elephant conflict. Ringling Bros. CEC assisted the DWC in the planning and execution of the First National Survey of Elephants in Sri Lanka in August 2011. It also carried out conservation education programs at several schools and colleges in the island. Students from Missouri State University in USA came over to the Ringling Bros. Center for the Study of Asian Elephant at Rajarata University in the summer of 2012 to observe wildlife (including elephants) and agriculture and to gain an understanding of the culture where elephants are a part of the landscape.
Introduction

The main goal of the Ringling Bros. Center for Elephant Conservation® (Ringling Bros. CEC) in Polk City, Florida, USA is to assist Sri Lanka in its efforts to conserve and manage elephants both in the wild and in captivity. To do so, it entered into partnership with the Universities and Government Departments. MOU was signed with (a) the Postgraduate Institute of Science – PGIS – of the University of Peradeniya, (b) the Sacred Temple of the Tooth – Dalada Maligawa in Kandy, (c) the Rajarata University of Sri Lanka in Mihintale, and (d) the Department of Wildlife Conservation.

Programs

Ringling Bros. CEC hosted four Sri Lankan graduate students for one year of training and research, utilizing the elephants at the Center, as part of their postgraduate degree program at the University of Peradeniya. After completing a year at the Ringling Bros. CEC in Florida, the students returned to Sri Lanka and continued their research projects on (i) reproduction, (ii) parasitology, (iii) behavior, and (iv) body condition of elephants in human care, under the joint supervision of Dr. Dennis Schmitt and Prof. Charles Santiapillai. Three of the students have submitted their theses to the University of Peradeniya for review by their MPhil committee for the degree of Master of Philosophy (MPhil), while the fourth student’s research was upgraded to PhD.

At the request of the Custodian of the Sacred Temple of the Tooth, a state-of-the-art bull pen was built at Pallekele, near Kandy (Sri Lanka) so that bull elephants in musth could be managed humanely in the absence of ropes and chains (Santiapillai et al, 2011).

The Ringling Bros. Center for the Study of Asian Elephant was built at Rajarata University, in Mihintale, to promote research and teaching in elephant conservation and management. The Center has an office, two chalets and a kitchen/dining area for the use of any visiting scholar. It is situated not far from a large seasonal population of free-ranging elephants. Ringling Bros. CEC has also established a fund to provide grants for research proposals for student investigations, with an emphasis on Wildlife Conservation at the Rajarata University. It also conducts a series of lectures on elephant management annually at which Prof. Raman Sukumar from the Indian Institute of Science takes part as well.

The MOU signed with the Department of Wildlife Conservation (DWC) has resulted in a number of initiatives.

Study of the growth rate of elephants

At the Elephant Transit Home (ETH) in Uda Walawe national park, the growth rate of elephants is being carried out at monthly interval for over three years. The elephant calves are weighed on a portable electronic scale (Wijeyamohan et al., 2010). The project also rendered service to the privately owned elephants which were weighed at regular intervals as well. Up to 35 elephants were weighed and the data on body weight was given to the elephant owners and the mahouts. However over 50% of the mahouts did not want their elephants weighed, perhaps due to superstition. The efforts of the staff at ETH to provide milk to and care for the elephant calves which arrived there as the result of many factors, is truly inspiring. The data from weighing these calves over their early life in human care is unique and will provide those caring for elephant in similar environments an invaluable reference.

Assessment of body condition of elephants

Wijeyamohan’s PhD research deals with the assessment of body condition of elephants, both in the wild and in captivity. He has developed a body condition scoring (BCS) system to monitor changes in body (Wijeyamohan et al., 2013 in preparation). Every elephant is photographed for future identification.
Assessment of the Human-elephant conflict

An assessment of the human-elephant conflict (HEC) was carried out across Sri Lanka in 2008, 2009 and 2011 in areas with a high concentration of elephants and agriculture. The surveys provide a record of the specifics of the various factors involved over time in HEC in those areas (Santiapillai et al., 2010). These surveys have identified HEC hotspots in the northwest and southeast of the island.

Morphometric measurements of elephants

An accurate method to estimate the height of elephants using laser beams was developed by Wijeyamohan et al., (2012). The technique has proved invaluable in obtaining linear body measurements of not only elephants but other wildlife as well.

Decibel levels of firecrackers

In association with the DWC, the University of Ruhuna and the University of Peradeniya, the decibel levels of the fire crackers that villagers routinely use to ward off wild elephants were tested. Results have shown that the decibel levels are indeed very high and hence they may impair hearing in elephants if they are exposed to them constantly (Sivakumar et al., 2013).

Tracking elephant movement

A collar that was designed and built by Wijeyamohan was used to track a bull elephant in the wild. A wild bull elephant was tranquilized on just standing sedation so that the home-made GPS/GSM collar could be attached to the animal in a few minutes. Subsequently the elephant’s movement was monitored on line once every minute for a month. This technology was mainly developed to monitor marauding elephants with the aim of mitigating the human-elephant conflict.

Care of elephant calves

In order to care for the injured or sick elephant calves, a treatment pen was built at the ETH in Uda Walawe national park. In addition, a much larger feeding station too has been built so that elephant calves could be fed with milk at regular intervals. The feeding station also enables the calves to be weighed at monthly intervals.

Conservation education

(a) A public address system was set up at the ETH so that the visitors (both local and foreign) could be informed about what is being done to rehabilitate and reintroduce the elephants to the wild. The conservation message is broadcast in English and Sinhala, while the Tamil version is being prepared.

(b) To promote conservation awareness among the public at large in Sri Lanka, the Ringling Bros. CEC initiated a Conservation Education Program at (i) Jaffna College, (ii) Uduvil Girls’ College in the Jaffna Peninsula, and (iii) at three under-privileged schools in the Vanni area where the human-elephant conflict was rife.

(c) Another program was carried out in Mannar through the Catholic Church at which about 40 priests took part.

(d) A public lecture on elephant conservation was delivered at the English Language Teaching Unit (ELTU) of the Faculty of Science at University of Peradeniya to about 500 students on 1 April 2013. Another lecture on the first national survey of elephants was delivered at the island’s
premier conservation NGO, the **Wildlife and Nature Protection Society of Sri Lanka** Colombo on 19 April 2013.

(e) At the request of the DWC, a conservation education program was held in Southern Sri Lanka in the vicinity of the Nilwala Ganga to educate both school children and government officials on the importance of crocodiles in the ecosystem and how to avoid coming into conflict with them. A conservation leaflet was published in English, Sinhala and Tamil for free distribution to the schools for which funds were given by the Ringling Bros. CEC program.

**Assessment of the status of elephants**

At the invitation of the Director-General of the DWC, the Ringling Bros. CEC assisted in the planning and execution of the **First National Survey of Elephants in Sri Lanka** in August 2011. It also provided 2000 souvenir bags and supported the publication of 1000 copies of the final report (Dissanayake *et al.*, 2012; Santiapillai & Wijeyamohan, 2012; Santiapillai & Wijeyamohan, 2013a). The survey has revealed the presence of a healthy, viable population of elephants with least skewed sex ratios. A minimum estimate of 5,879 elephants was recorded.

**Miscellaneous**

The Ringling Center also donated the following items: (a) pairs of shoes and T-shirts to the 30 field staff at the ETH; (b) a high pressure water pump to clean the elephant holding area; (c) a computer to the ETH office; (d) funds to defray the travel cost of Dr. Vijitha Perera – the resident veterinarian at ETH - so that he could attend the conference of the International Elephant Foundation at Rotterdam, in The Netherlands in 2011 and another Conference on Veterinary Medicine at Salt Lake City, USA in 2013.

**International Symposia**

Scientific papers based on the research carried out under the Ringling Bros. CEC program were presented at the meetings of the Elephant Managers Association (EMA) in Orlando, FL (2008), Houston, TX (2009), Pittsburgh, PA (2010), Rochester, NY (2011) and Santa Barbara, CA (2012). At the invitation of the University of Canterbury in Christchurch, New Zealand, two papers were presented at the **International Symposium on Human-Elephant Relations in South and Southeast Asia** in May 2013 (Wijeyamohan & Santiapillai, 2013; Santiapillai & Wijeyamohan, 2013b).

**International Student Study Program**

A short term study away course was provided in the summer of 2012 in Sri Lanka for two weeks at which three undergraduate students from the Missouri State University in Springfield, MO took part under the leadership of Dr. Dennis Schmitt. The students followed lectures and were able to observe elephants, other wildlife and agriculture to gain an understanding of the culture where elephants are a part of the landscape. Plans are being discussed so that a student exchange between the universities of Missouri State (USA) and Rajarata (Sri Lanka) for study away programs will continue and expand.

**Conclusion**

These are just some of the projects that are being supported by Ringling Bros. CEC in Sri Lanka through its **Center for the Study of Asian Elephant at Rajarata University of Sri Lanka at Mihintale, Sri Lanka**. It is Ringling Bros. CEC’s desire to continue as a long-term partner for Asian elephant conservation with Sri Lankan Universities and the Department of Wildlife Conservation.

**Acknowledgements**
We would like to thank Mr. Kenneth Feld, Chairman & CEO, Feld Entertainment, Inc. for his continued financial support. We acknowledge with thanks the support given to the program by Mr. Kirk McCoy, Vice President, Human Resources at Feld Entertainment, Inc. Our thanks to the University of Peradeniya, the Rajarata University, and the Department of Wildlife Conservation for their assistance.

References


Control of Invasive
Arenga Palm (*Arenga obtusifolia*) in Habitat Suitable for Javan Rhino (*Rhinoceros sondaicus*) in Ujung Kulon National Park

By: Sectionov Inov, IRF Indonesia Liaison

2013 International Elephant & Rhino Conservation & Research Symposium
Javan Rhino

World Population ~40
Distribution of Arenga Palm in Ujung Kulon National Park
(Based on Landsat ETM Imagery of 7th August, 2008)

Legend
- Red: Distribution of Arenga Palm
- Green: Ujung Kulon National Park

Map Prepared by Aaranyak in Partnership with YABI & IRF.
Why Arenga Palm

- Considered as invasive species
- Not used intensively by the Rhinos
- Overshadowing inhibits growth of other plant species (reduced biodiversity)
Main Ideas

- Prevent any increase/reduce the distribution of *Arenga obtusifolia* within Ujung Kulon National Park;
- Increase natural feeding grounds commonly used by Javan rhinos;
- Document Javan rhino habitat utilization pre-and post-injecting and cut down of palms on experimental plots; and
- Evaluate the most cost-effective and environmentally-responsible techniques for habitat restoration.
Methodology

Manual (Cutting Tree)
Injection Herbicide (*glyphosate*) Treatment
First Monitoring

Seedling growth

Before
After

- Langkap
- Rotan seel
- Jajambuan
- Salam
- Sulangkar
- Areuy Gadel
- Kedondong
- asahan
Seedling growth After cutting treatment
Injection Treatment and result after 6 month
Mortality Rate Arenga Palm

Mortality Rate

Ho July 2011

H1 Oct 2011

H2 Feb 2012

0%
82%
100%
120%
The dominant factors affecting palm clearance and re-growth patterns are seasonal weather patterns, light intensity and methods of seed dispersal.

Chemical clearance methods (the injection of glyphosate isoprophylammonium © Roundup), produces relatively rapid palm mortality (three months), produces no detectable negative environmental impacts, and is no more expensive than cutting.
Discussion

- By comparison, manual palm clearance (cutting and removing trunks, fronds and fruits) is essentially immediate (about one week to clear one hectare), but requires a larger local work force and thus engages more members of neighboring communities in this wildlife conservation effort.

- Preliminary results document a significant rate of plant regrowth on experimental plots, a predominance of rhino food plant species (more than 90%) replacing areas initially covered by *Arenga obtusifolia*, and an apparent increase in restored habitat use by the resident Javan rhino population.
Thank you
The role of standing sedation in mitigating the human-elephant conflict in Sri Lanka

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Abstract

With the introduction of the commercial dart in 1953, chemical immobilization of wildlife including elephants became a routine management practice. It was in 1967 that the staff of the Department of Wildlife Conservation (DWC) in Sri Lanka was first introduced to the use of the drug M-99 as a means of anaesthetizing elephants. However, such immobilization has its own risks where the elephant can injure itself or die while being anaesthetized. By contrast, standing sedation using Xylazine (Xylazine hydrochloride) is safer for the elephant and the effect can last longer and be utilized more often than anesthesia. A home-made collar was fastened around the neck of a wild bull elephant using just a padlock and chain instead of the usual nuts and bolts for easier and quicker attachment to the elephant after it had been tranquilized under standing sedation. The transmitting GPS/GSM unit comprised of 1.6 kg 100 Ah rechargeable battery to signal the location of the elephant once every four minutes. This allowed us to monitor the elephant online in real-time. The software used is quite versatile to establish geo-fencing where e-mail or SMS alerts could be sent to mobile phones. Thus, immediate action is possible to chase the elephant back into the forest before any catastrophe occurs. The software also has the capability to monitor remotely the battery level. As the battery is rechargeable, the elephant could be brought under standing sedation to replace the old collar with a new one for continuous monitoring. Online monitoring also reveals daily behavioral patterns such as patterns of utilization of habitats, the number of attempts the animal makes to raids crops and fine-tuned movement patterns including resting times and the distance traveled each day.

Introduction

Sri Lanka with a total land area of 65,610 km\textsuperscript{2} and a human population of c. 22 million, is one of the most densely populated island having a crude density of 352 people per km\textsuperscript{2}. Despite its small size and relatively high human population, Sri Lanka supports an elephant population estimated to be in excess of 5,879 or at a density of 0.1/km\textsuperscript{2}. (Dissanayake et al. 2012, Santiapillai and Wijeyamohan 2013).

Almost a quarter of the country is under forest while 14\% of the land area is protected by the Department of Wildlife Conservation (DWC) under the Flora and Fauna Protection Ordinance. However, the total range of the elephant in the country extends across almost 50\% of the land area. This gives a crude density of 0.2 elephants per km\textsuperscript{2}. The crude density of elephant increases further to as much as 0.7/km\textsuperscript{2} if only the 14\% of the protected areas (Fig.1) are taken into consideration. An elephant density of 0.7/km\textsuperscript{2} would represent about the highest among the Range States in Asia. Given this situation, it is not difficult to appreciate why the human-elephant
conflict (HEC) is inevitable. As the result, annually, almost 50 people are killed by wild elephants and between 100-180 wild elephants perish in the conflict.

Fig. 1. The network of Protected Areas under Department of Wildlife Conservation (DWC) in Sri Lanka

Although Sri Lanka has tried several methods in the past to mitigate the HEC, its resolution still remains elusive. Along these lines, we propose yet another solution to mitigate the HEC by making use of modern technology.

**Methodology**

The technology includes sedation using Xylazine (Bongso 1979) and fixing a collar equipped with GPS/GSM while the elephant is still standing (Alfred et al. 2012). The collar was home-made comprising of a 75 mm rubber canvas belt with a box containing the GPS/GSM unit and two 50 Ah (Tenergy) batteries imported from Taiwan and the USA respectively.
In order to minimize the fixing time of the collar around the neck of the sedated elephant, chain links were attached at the both ends of the rubber canvas belt. Strong padlocks were used to fasten the belt and the box to the belt. The box with the GPS/GSM unit and the batteries was designed to hang like a pendent below the neck. As the chain links were attached at the end of the belt, it was possible to adjust the belt according to the circumference of the neck of the elephant. The entire collar weighed about 12 kg which is well under 2% of the total weight (Brown et al. 1999, Jepsen et al. 2005) of over 4000 kg elephant.

The GPS unit gave very accurate location (within 1 to 5 m radius) which was transferred to the GSM section of the unit at every 4 minute interval. The GSM unit used a local cell phone network to transmit SMS. Information sent via SMS is instantly visible on the Google map used by the software provider. As the data was updated every 4 minutes, the collared elephant could be monitored online in real time.

Standing sedation

Standing sedation on wild elephants had been carried out successfully on several occasions in Sri Lanka. However, no such sedation was ever undertaken for the sole purpose of collaring an elephant. A successful sedation and collaring was carried out on August 24, 2012 on a wild bull elephant named Wanaraja (or King of the Jungle) at the Uda Walawe national park. The principal veterinarian, Dr. Vijitha Perera identified the bull elephant at 0827 hrs for sedation. The bull was about 25 years of age and in good body condition, with a score of 6 (on a scale 1-emaciated to 10-obese) although it had a gunshot wound on the lower right foreleg at the distal end of the radius. It was about 2.7 m in height (at the shoulder).

The first dart containing 5 cc (500mg) of Xylazine hydrochloride (Chanazine®) was fired by Dr. Perera at 0910 hrs and the bull immediately fled into the nearby forest from the open grassland where it was feeding. A second injection of the drug 3 cc (300mg) was administered by hand at 0932 hrs once the animal was located in the forest. By 0950, the animal began to show symptoms of the impending standing sedation and stopped moving. Other signs include the ears which began to slow down their rate of flapping and subsequently became almost stationary, perpendicular to the body axis; the tail and trunk too ceased moving but became relaxed; and the penis protruded from the prepuce. By 0955 hrs the bull began to snore lightly. We tested the awareness of the bull by throwing a stick at it, and when there was no response, it was the signal to move in and fix the GPS collar around the elephant’s neck at 0957 hrs. The collar was securely fastened using three padlocks. The entire operation from darting to collaring took 50 minutes (Fig.2.). At 1006 hrs 6cc Yohembine hydrochloride (60mg) was administered through the saphenous vein on the right hind leg. By 1014 hrs the ears and the tail of the bull started to move, and by 1016 hrs the animal began to move its legs. By 1025 hrs, the elephant moved into the forest and its movement could be monitored on a hand-held smart phone.
Advantages of the technique

The collar was fixed with two 50 Ah rechargeable Li-Fe polymer batteries. The box consisting of the GPS/GSM unit with the batteries was made in such a way that the batteries could be recharged upon recovery. Thus the collar is reusable. According to Bongso (1979), repeated administration of Xylazine for sedation even as much as seven injections per animal at intervals of three to four days had no adverse effects on Asian elephants.

The collar that we built worked for 32 days. Furthermore, the system has the facility to inform the users when the battery reaches 20% of its capacity. During our experiment, although we received the message concerning the drop in battery charge to 20%, the battery was left to run until it was fully drained mainly to monitor how long the battery would still function even after reaching 20%, which amounted to a total of 12 days. This provides us ample time to locate the animal and change the collar. Such a window of time would give us the opportunity to locate the elephant and replace the old collar with a new one. The old collar could then be recharged for the next operation.

Since the battery drained completely, we were unable to monitor the bull on line. Hence there was an active search on the ground by the DWC personnel. Thus, the collared elephant Wanaraja was subsequently located and sedated on March 8, 2013 when the old collar was removed (Fig.3.) and replaced with a new one. The collar remained on the elephant for little more than 6 months. The elephant had no wounds or injuries on the neck or elsewhere.
The software that comes with the system has facilities to establish geo-fences electronically. Geo-fences can be created around protected areas and villages with buffer zones in between. The moment elephant having the collar crosses any of these geo-fences, it will alert the users via SMS or e-mail. From then on, managers can monitor the movement of the elephant using an Internet browser on a mainframe computer, laptop or smart phone. If the elephant is moving towards a village or cultivated area, a team could be dispatched immediately from the nearest location to chase the elephant back into the forest. Since sedation using Xylazine is safe on elephants (Bongso 1979), the identified marauding elephant can be kept away from people and property for any long time until the elephant learns not to do so.

**Application**

This method has other advantages as well. Sometimes, after the removal of an identified marauding elephant from an area, people have reported that crop raiding was still going on. In such instances, the villagers tend to blame the authorities for removing the wrong elephant. Therefore, if our collar is deployed on an alleged crop-raider, managers can study the behavioral pattern of that elephant and confirm whether or not it was the trouble maker. Studies also can show whether the elephant is a deliberate crop raider or an accidental intruder.

After collaring Wanaraja on August 24, 2012 we found that his movement was getting reduced day by day. Therefore we decided to check him out on September 10, 2012, and were able to track the animal inside the Uda Walawe national park at 1400 hrs. To our horror we saw the animal lying on its side on open grassland under a blistering sun. At first we feared for the worst and thought that the animal may have died. However, once we saw the tail twitching we were relieved to know that it wasn’t so. When we moved closer the animal got up and started feeding on the dry grass. Dr. Vijitha Perera however noticed that the animal was walking with a slight limp.
and observed an infected old bullet wound on the right foreleg towards the distal end of the radius. Immediately he decided to do a standing sedation and gathered his team and equipment.

Dr. Perera and his team decided to clean the wound and thoroughly washed it with normal saline and cleaned with Povidine iodine. Mixture of Povidine iodine, Coumaphos, Propoxur and Sulfanilamide applied. Finally antibiotic and multivitamins injections were given. Throughout the entire operation, the elephant remained sedated and could be approached and treated. Subsequent monitoring of the elephant indicated that his movement had increased day by day. Similarly, when wild elephants are seriously ill or wounded our collars can provide a means for continuous monitoring and treatment until they recover completely. A first time treated elephant in the wild could be fixed with this collar for online monitoring and to locate it for subsequent treatment. As the collar is rechargeable, every time the team goes to treat the elephant, it can be replaced for charging afresh.

Discussion

In the past, the movement of wild elephants had been monitored using VHF collars whose signals were picked up by a hand-held directional antenna. However such transmitters had a range of only 3-4 km and the position of the elephant was located through triangulation. This is an extremely labor-intensive operation which nevertheless gives only 4 or 5 data points per day (Fernando et al. 2008). Such a technique was subsequently improved with the use of a GPS unit that tracks an elephant within a few meters of accuracy via a network of satellites (Blake, Douglas-Hamilton and Karesh 2001). This system would deliver just 6 data points to identify the positions of the collared elephant in 24hrs (Fernando et al. 2012). The drawback of this technology is that no one will know where the elephant had gone in between successive data points during the 4hr interval. In the meantime it would be equally difficult to re-locate the elephant in the field because by the time one gets to the last data point which was sent 6 hours earlier, the elephant would have moved on. Furthermore, a 6-hr interval is more than enough for the elephant to raid crops and get back to where it was and no one would have guessed what had indeed happened. Furthermore, the technology would just give the position of the animal and nothing about its activity pattern or movement.

Chemical immobilization of wild elephants is not a new phenomenon in Sri Lanka. One of the ways in which elephants were captured in the wild in the distant past had been through the provision of opium via fruits place along elephant trails. The morphine in the opium made the elephants sedated enough to let the elephant catchers approach and noose them. In Africa, elephants had been immobilized and killed for food by natives using poisoned arrows (Fowler 2006). With the introduction of the commercial dart in 1953, chemical immobilization of wildlife including elephants became a routine management practice. It was in 1967 that the staff of the Department of Wildlife Conservation (DWC) in Sri Lanka was first introduced to the use of M-99 as a method of tranquilizing elephants involved in crop depredation by Gray and Nettasinghe (1970) who noticed that the requirement of the drug was approximately twice that for the African elephant.

In the past, the standard practice in the capture of wild elephants in Africa and Asia, be it for treatment, tracking or translocation, had been to first anaesthetize the animal concerned through the subcutaneous administration, via Cap-Chur darts, of such powerful analgesic drugs as Etorphine hydrochloride (M-99) and combination of Etorphine hydrochloride and Acepromazine
maleate (Immobilon) and then using an appropriate antidote such as Diprenorphine hydrochloride (M5050 or reverzine) to reverse the effects. However, such tranquilization has its own risks and can be dangerous both to the elephants as well as the members of the darting team. The tranquilized animal can injure itself or may die under anesthesia. The risks are particularly high in areas where the elephant density is high and vegetation is thick and thorny. Furthermore, because of its huge size, once an elephant is tranquilized, measures have to be taken to revive him as early as possible. If an elephant is kept under anesthesia for a long period, it could die. Hence the darted animal must be located as early as possible – not an easy task in the dense and tangle vegetation that is so typical of elephant habitat in Sri Lanka. Furthermore, once the elephant is located, every effort must be made to ensure that it is in a suitable lateral position and also in a stable anesthetic state. If the elephant lies on its sternum, it must be pushed over onto its side, to ensure that it is in the lateral recumbency and thereby avoid respiratory problems. Such complete anaesthetization of elephants cannot be recommended in swampy areas or in habitats close to aquatic ecosystems to avoid death from drowning. Thus repeated complete anaesthetization in short period of time is very risky.

Our approach to tranquilizing wild elephants using standing sedation offers a safe and secure means to tranquilize any elephant. It is less dangerous than conventional tranquilization and provides a safe method to treat or track elephants in the wild. Studies shows that VHF or GPS collars does not interfere with the behavior of the elephants (Horback et al. 2012). In this context, the new technology described here provides a very valuable method to monitor the movement of elephants during day and night, irrespective of the weather, on line from anywhere in the world. As long as the elephant is moving, its movement can be monitored on a computer and daily movement pattern could be recorded to assess its true home range. It will provide useful information on how much time the elephant spends on feeding, drinking and resting or sleeping with reference to a Google map of the area. This technology of monitoring elephants online could be used to treat elephants in the wild. The technology described in this communication has never been tried in Asia to the best of our knowledge. We also believe it will supersed all previous methods of monitoring elephants in the wild.

References


Temporal and spatial patterns of human-elephant conflict in Nepal

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This study addresses spatiotemporal patterns of Human Elephant Conflict (HEC) in Nepal by reviewing available historical records published electronically in 9 daily national newspapers over a 10 year period (2003-2012). Over the past decade, HEC has caused 100 human deaths, 47 serious human injuries, and 615 cases of extensive property damage; additionally, there have been 16 elephant deaths and 6 severe elephant injuries. Data were analyzed using regression and χ² tests to investigate temporal and spatial patterns of conflict. HEC intensity was highest in the migratory route along the eastern Indo-Nepal border region, and increased across the time period reviewed. HEC is elevated during the winter months, at night, and during the rice harvest season. Human casualties are biased towards males and individuals aged 40-70. Possible mitigation measures recommended specific for Nepal include more effective fencing around protected parks, development of corridors between patchy forests, and reallocation of resources derived from the tourist industry towards conservation initiatives.

Key words: Asian elephant, human-elephant conflict (HEC), Nepal, spatiotemporal analysis, Terai
Asian elephants (*Elephas maximus*) are receiving international attention as they are recognized as an endangered species by the IUCN red data book. Historically, Asian elephants had a range that included approximately 9 million km$^2$ encompassing much of South Asia, from West Asia to Southeast Asia; their present range represents 5% of that historically found, extending from South to Southeast Asia (Choudhury et al. 2008). In Nepal, the topography limited elephants historically to a narrow southern strip of the lowland Terai ranging in width from 10 – 50 km (Fig 1a); deforestation and land use practices have further reduced their range in Nepal (www.iucnredlist.org).

The Terai consists of river valleys and low altitude hills (70m - 700m). Historically, marshes, forests, and high levels of mosquito infestation associated with malaria comprised these lowland valleys (Gallup and Sachs 2000). Land use practices over the past 50 years have resulted in the draining of these lowlands, increased pesticide use, and conversion of wild native grasslands and forests to highly productive farmland. Several crops are alternated annually, including wheat, rice, and maize. Associated with this agricultural land use has been a 3-fold increase in human populations in the last 50 years (GoN/MoHP 2011). Within segments of the Terai, human density (330 individuals per km$^2$) is the highest among the physiographic regions of Nepal (GoN/MoHP 2011).

These changing land use practices and increasing human densities have resulted in loss of habitat for a highly migratory species. In addition to overall loss of habitat, habitat fragmentation, degradation and loss of connectivity between elephant habitats have occurred in Nepal (Yadav 2004; Shrestha 2007) and throughout South Asia (Sukumar 1989; WWF 2006; Cordingley 2008; Fernando et al. 2009). As a result, human elephant conflict (HEC) has become common, and is the single greatest threat to the survival of Asian elephants.
Human-Elephant Conflict in Nepal

throughout their range (Sukumar 1989; Choudhury et al. 2008), resulting in a critical conservation problem (Fernando et al. 2009). HEC results in human property damage, including significant loss in agricultural production from crop damage. For example, Shrestha (2007) estimated a 25% local crop loss in parts of Nepal due to elephants. More serious are the human deaths and injuries caused by HEC. Elephants are in turn killed both for protection from, and in retaliation to, these attacks on humans and property. Conservation needs and peoples' interests are in direct and often violent conflict in regions of HEC. The survival of the Asian elephant throughout its range is further impeded by socio-economic and political conditions of the countries where conflict exists, as few resources are available to address these issues and human densities continue to increase (Sukumar and Santiapillai 2006).

HEC records indicate that South Asian countries are facing the highest number of casualties (defined as deaths and severe injuries) among the nations inhabited by Asian elephants. For example, India has recorded 300 human and 200 elephant deaths annually, whereas Sri Lanka experiences 50-70 human and 150 elephant deaths per year (Parera 2009). If rates of elephant deaths in Nepal are comparable relative to their population size, with an estimated residential elephant population of 109-142 (DNPWC 2008), then significant loss of elephants could lead to extirpation in this country. However, data are lacking regarding the monitoring of elephant attacks on humans and elephant mortality in Nepal, although some research has assessed economic losses due to crop damage from elephant movements (Yadav 2004; Shrestha 2007).

Therefore, the purposes of this study were to determine the magnitude of HEC in the Terai of southern Nepal, and to document spatiotemporal patterns and characteristics of HEC.
These incidents were then evaluated in the context of social and demographic variables of the victims. Thus, this study provides government, conservation agencies, and researchers with concise and up-to-date information on the patterns of HEC in Nepal, and a discussion of practical management options to effectively mitigate HEC.

**Materials and Methods**

*Study area.* — Nepal, one of the most densely populated countries in South Asia, is a landlocked, mountainous country. Geographically, Nepal is bordered by China to the north whereas India borders the remaining perimeter. About 80% of the inhabitants of the Terai depend on agricultural subsistence farming (GoN/CBS 2010). The Terai is known as the breadbasket of Nepal with a dense human population, a coexistence of large mammals such as the Asian elephant, one-horned rhinoceros (*Rhinoceros unicornis*), and Bengal tiger (*Panthera tigris*), patchy forests, and subsistence farming. Within this region there are 6 protected parks, fragmented forests and three primary trans-border migratory routes for elephants (Fig. 1a). Patchy forests typically range in size from 100 – 1000 ha in size and serve as temporary refuges for elephants and other wildlife.

Two types of elephant herds occur in the Terai: residential elephant populations that are typically small in size (5-15 individuals per location), and larger trans-border migratory herds (20-100 individuals per location), concentrated close to the Indo-Nepal border (Yadav 2004; Pradhan et al. 2007, Shrestha 2007). The eastern and western regions contain trans-border migratory routes (Velde 1997; Yadav 2004; Shrestha 2007); elephants within the central region are considered to be residential only (DNPWC 2008).
Methods. — The southern belt of Nepal was divided into 3 study regions (east, central, and west) for the purposes of this study, based upon the location of protected areas, the presence of elephants, and historical reports of elephant movements within these regions (Kharel 2002; Shrestha 2007, DNPWC 2008; Pradhan et al. 2011). There are 3 parks located within the western region, 2 parks in the central region and a single smaller park in the eastern region (Fig. 1a). The western and central regions have extensive electric fencing to protect crops from elephants moving between the parks and the buffer zones whereas the eastern region has very little electric fencing. Districts neighboring the Terai were also included if there were historical HEC events.

Variables studied were similar to those examined by Wakoli and Sitati (2012) for African elephants (Loxodonta africana) in a single district of Kenya. There is a lack of a coordinated national mechanism for the reporting and record-keeping of HEC in Nepal. Therefore, quantitative analysis of HEC is difficult due to the limitations in collecting comprehensive and detailed data (Gupta and Nathawat 2009). Nonetheless, as human injury and death as a result of elephants is sensational news, the best source of information is that reported in newspapers. We studied records of HEC that were electronically published over a 10 year period (2003-2012) in 9 daily national newspapers (Annapurna Post, Kantipur, Nagarik, Naya Khabar, Nepal News, Republica, the Kathmandu Post, the Himalayan Times, the Rising Nepal). The Nagarik and Republica were available online beginning in 2009, whereas the other newspapers were published each of the 10 years. Redundant incidents among newspapers were consolidated so as to provide as much information as available, while not “double counting” events. Limitations of using newspaper stories are the lack of public interest in, and therefore under-reporting of, crop and typical property damage by
elephants. Most house and property damages were also not published due to a lack of reporting by victims; there is no insurance or governmental compensation for property damages caused by wildlife. Thus, we likely only have partial information on those variables available and no statistics were performed on these data. We consider data reliability to be greater for the reporting of human casualties, elephant deaths, and extensive property damage resulting from HEC.

Other variables reported in the newspapers, and therefore of use in better understanding temporal and spatial trends of HEC, include information provided for the victims such as sex and age of the attacked person, time of day and dates for the incident, sex and age of the elephants killed, and the number of elephants observed. Days were divided into morning (03:01 – 06:00), daytime (06:01 – 17:00), evening (17:01 – 20:00) and nighttime (20:01 – 03:00) based on daily activities of local residents.

Statistical analysis.—HEC resulting in deaths and severe injuries of elephants and humans was also compared relative to season and crop type. Nepal has 3 climatic seasons: the monsoon season, which typically starts from the middle of June and ends during late September, the cold drier winter season from October to January, and the warmer drier spring season, from February to May. Based upon agricultural practices, months were categorized into 4 cropping seasons which are rotated during the year: wheat (January-March), maize (May-July), rice (September-December), and a non-crop season (April and August), where most of the croplands are fallow. Harvest is associated with the last month of each cropping season.

Linear regression analyses were used to study the changing frequencies of HEC and human casualties over time. Chi square tests were performed to compare human or elephant
deaths or property damage relative to geographic region, season, time of day, and crop rotation. Chi square tests were also used to reveal potential heterogeneity in frequency of HEC in terms of human victims’ gender and age. Human age was categorized by 10 year intervals. Alpha levels were set at 0.05 for all significance tests.

**RESULTS**

In the past decade, there were 239 articles in the nine national daily newspapers covering elephant-inflicted damage. From those reports, 615 houses were damaged by elephants, which, as stated, above, is likely an underestimate. HEC resulted in 100 (annual $= 10.0 \pm 2.3$) human deaths, 47 (annual $= 4.7 \pm 1.5$) human injuries, 16 elephant deaths (annual $= 1.6 \pm 0.4$) and 6 elephant injuries all of which were reported in eastern Nepal from a single event in 2007 (Table 1). Causes of elephant mortality were gunshot ($n = 7$), electrocution ($n = 5$), machete ($n = 1$), and 5 cases in which the cause of elephant death was unidentified.

Most elephants were killed when only one elephant or small herds ($< 10$ individuals) were engaged in HEC. Only 5 of the dead elephants were identified by gender, of which 3 were females and 2 were males. Similarly, 6 of the dead elephants were identified according to age: 4 calves (age 1-4 years) and 2 adults (age 40+ years).

*Temporal distribution of HEC in Nepal* — Regression of HEC in Nepal from 2003-2012 indicated that HEC incidents have increased across the study period with an association between year and number of HEC incidents (Fig. 2; $Y_t = -7.27 + 5.39*t$; $F_{1, 8} = 28.07$, $p < 0.001$, $R^2 = 77.8\%$). Consistent with the above, the number of human casualties has also significantly increased over the past year, with an association between year and number of human casualties (Fig. 2; $Y_t = -2.73 + 3.17*t$; $F_{1, 8} = 18.99$, $p = 0.002$, $R^2 = 70.4\%$).
HEC incidents occurred year-round but were more frequent from September to January (Fig. 3). This period coincided with the end of the monsoon season and the winter. HEC was heterogeneous among seasons, with higher frequencies in the winter (Fig. 3; $\chi^2 = 82.56$, df $= 2$, p < 0.001). There was also a significant difference in frequency of HEC relative to crop rotation (Fig. 3) ($\chi^2 = 117.22$, df $= 3$, p < 0.001). HEC cases were negligible during times when no crops were being raised and greatest during the harvest of rice (Fig. 3).

Human casualties occurred year-round but were highest during the winter (Fig. 4). In contrast, most elephant deaths occurred during the monsoon period (June and July; Fig. 4). Property damages were greatest in the monsoon and winter periods (Fig. 5). Among the human victims, males (61.9%) were killed more frequently than females (38.1%) ($\chi^2 = 6.64$, df $= 1$, p = 0.01). Mature individuals were attacked more frequently than were elderly and younger individuals (Fig. 6; $\chi^2 = 18.43$, df $= 7$, p = 0.01). There was a particularly high incidence for individuals between the ages of 40 – 70 years. Children under the age of 10 were also frequently killed. There was a significant difference in the incidence of HEC relative to the time of day, with almost two-thirds of all incidences occurring during the night (Fig. 7; $\chi^2 = 133.63$, df $= 3$, p < 0.001). Most males were killed during the night whereas females were more often killed during the daytime.

Spatial distribution of HEC in Nepal — There was heterogeneity in the incidence of HEC relative to geography in the Terai of Nepal with an increase in incidence in eastern Nepal relative to the central and western regions (Table 1; $\chi^2 = 77.93$, df $= 2$, p < 0.001). The highest incidence of HEC occurred within the corridor at the extreme eastern border with India, followed by the buffer zone around Chitwan National Park in central Terai, the buffer zone around the Koshi Tappu Wildlife Reserve in eastern Terai, and equal incidences in the
buffer zone around Bardia National Park (western Terai) and a patchy forest zone in eastern Terai (Fig 1b).

When elephants were directly observed during HEC, most incidents (69%) involved a single elephant. The number of elephants involved in HEC was highly variable, with the larger herds occurring in eastern Terai. For example, a single report in eastern Nepal estimated 150 individuals, whereas a second report estimated 80 individuals, and 4 reports indicated that a group of elephants were involved. There were also several larger herds observed in western Nepal, where 6 incidents reportedly involved between 10 - 30 elephants. For central Nepal, only a single report indicated 10 or more elephants involved in the HEC. For eastern Nepal, 8 of 10 instances when herd sizes were observed to be 10 or greater occurred during the monsoon season. In contrast, in western Nepal only 2 of 6 cases of herds of 10 or greater causing damage occurred during the monsoon.

**DISCUSSION**

Over a 10-year period, HEC has resulted in 147 human and 22 elephant casualties in Nepal. In contrast, Yadav (2004) identified 66 human deaths and 17 elephant deaths in eastern Nepal from 1986 - 2002. Shrestha (2007) also identified increasing HEC from 1999 - 2007. The numerical trends of these 2 studies provide evidence of increasing conflict intensity. Although some studies have investigated HEC at local levels in Nepal using human surveys (Yadav 2004; Shrestha 2007), ours is the first systematic approach to identifying the spatiotemporal distribution of human-elephant conflict at a national level in Nepal. Several studies measuring HEC have been performed in African countries by field researchers (e.g., Kiiru 1995; Ngure 1995; Maingi et al. 2012). However these data are not
collected and tabulated by the government of Nepal; thus an indirect measure of HEC by way of newspaper articles was performed in the present study.

Fernando et al. (2005) identified HEC as the greatest threat to the survival of Asian elephants. The increasing trend of HEC in both India (Choudhury 2004) and Nepal (this study) is associated with increasing human densities in historically undeveloped areas. As habitat is transferred from wetlands and native forest to croplands, humans and elephants come in more frequent contact, and the effects thereof are exacerbated. The number of deaths of both humans and elephants is much lower in Nepal than that of both India and Sri Lanka (Parera 2009), yet over a much smaller area. Despite these lower total numbers of deaths for Nepal elephants, the levels we report are still alarming. With an estimated residential herd of less than 150 individuals (DNPWC 2008), the loss of 16 individuals for a species with low fecundity is likely a significant loss.

It is not known at present whether those elephants killed were residential or migratory, as elephants move independent of national borders. The highest incidences of HEC occurred in the eastern corridor where migration has historically been common. Herds greater than 100 in number have been observed moving through this corridor (this study; unpublished data; DNPWC 2008). Additionally, buffer zones around protected areas had high rates of HEC. Bardia National Park has the greatest residential herd, with estimates of 80 (Pradhan et al. 2007). Additionally, a well-maintained corridor facilitates the movement of elephants between Bardia and India. Chitwan National Park and the adjoining Parsa Wildlife Reserve have an estimated population of 20 - 30 elephants (DNPWC 2008). The number of residential elephants in Koshi Tappu Wildlife Reserve is quite small (n = 7 – 15), although numbers have been difficult to estimate due to elephant movement patterns within this
region (DNPWC 2008); therefore, HEC associated with this zone is probably due to elephant movement along the eastern region of the Terai. Further, patchy forests as found in eastern Terai have no residential herds and served to enhance elephant mobility, fostering HEC. The herd size of Shuklaphanta Wildlife Reserve is also quite small (n = 3 - 5; DNPWC 2008) and has a corridor connecting with India. HEC in this area is limited. Further research on population structure is required to understand the interactions of resident and migratory elephants of this region, as well as which of these elephant groups are involved in HEC.

Most HEC occurred during the dry season of winter followed by monsoon season, consistent with that found in a previous study of Nepal (Shrestha 2007) and in Sri Lanka (Ekanayaka et al. 2011). Most of the elephant damage occurred during nighttime or early morning, similar to that found by Shrestha (2007). A potential explanation for this pattern may be that elephants usually leave the forested areas for crop raiding at night when human activity and intervention is lowest (Wakoli and Sitati 2012). Nighttime invasion would also result in the higher mortality of both humans and elephants observed in the present study, largely as a result of greater confusion and poor visibility. Most of the victimized people were mature males between the ages of 40-70 years. Males guard their cultivated lands at night (Sukumar 2003), which results in a higher chance of encounter to elephants. In contrast, females have a higher mortality from elephants during the day as they collect firewood and fodder from the forests where the elephants are residing during daylight hours. The mature age structure of those males attacked (40-70 years) may be due to the changing demography of the region. Many younger males leave the villages at an early age to work in Middle Eastern and Asian countries where there are better employment opportunities. Over
half of households in Nepal have a family member working in a foreign country (GoN/CBS 2011).

In contrast to much of the HEC present in Africa (Barnes 1996; Parker et al. 2007) and even in India (Choudhury 1999; Datta-Roy et al. 2009; Parera 2009) where poaching of elephants for their tusks has become severe, much of the HEC in Nepal is initiated by elephants. Two reasons for the lack of elephant poaching in Nepal are a strong cultural and religious foundation for revering elephants (Kharel 2002) and a strong military presence in areas where poaching large mammals has historically been problematic (Martin and Vigne 1996; Heinen and Shrestha 2006).

HEC Relative to Crop Production — Crop raiding by and resultant retaliatory killing of elephants have a long history for both Asian and African elephants (Lahm 1994; Choudhury 2004). HEC events in Nepal (Shrestha 2007; this study), similar to that of India (Choudhury 2004), are most associated with the harvest of rice in the winter months. Rice represents a rich energy source for elephants and harvest loss from elephants can be devastating to farmers (Choudhury 1999). Elephants often break down walls and enter houses in Nepal and can eat hundreds of pounds of harvested rice in a single evening (D. Neupane, personal observation). Such a single event may represent the loss of an entire harvest of a family farm. Choudhury (2004) observed elephants migrating to and congregating in the adjacent forests during the rice-growing season in India.

HEC occurred at lower frequencies during the growing of maize and less so for other crops, similar to that found by Shrestha (2007). Other studies have shown a large overlap of human crops and elephant diets, and that elephants consume a wide variety of crops (Sitati et al. 2003; Yadav 2004; Fernando et al. 2005; Campos-Arceiz et al. 2009; Ekanayaka et al. 2009).
284 Additionally, damage is typically greatest when the crops are mature (Sukumar 1990; 
285 Campos-Arceiz et al. 2009; Ekanayaka et al. 2011), similar to what we observed. Asian 
286 elephants apparently prefer rice to natural foodstuffs rather than feeding on crops as a result 
287 of natural food shortages (Ekanayaka et al. 2011). It has been suggested that the feeding of 
288 elephants on maize during the monsoon season of Nepal and other countries may be partly 
289 due to declining food quality of native vegetation during that time (Sukumar 1989; Osborn 
290 2004; Shrestha 2007). 
291 Alternative cropping has been recommended to reduce HEC (Yadav 2004). During 
292 interviews of villagers in Nepal, Neupane (unpublished data) found that elephants did not 
293 utilize tea plants as food; in contrast, the elephants tended to avoid areas where tea was 
294 being cultivated. Ekanayaka et al. (2011) identified 4 other cultivated species which were 
295 not predated upon by elephants in Sri Lanka, including chili, peanuts, onions, and sesame. 
296 However, rice represents a significant part of the local economy in Nepal, contributing 25% 
297 of the national gross domestic product (MoAC 2005). The decisions to substitute rice and 
298 other crops with alternative crops must be made with a cost-benefit approach for an 
299 agriculture-based economy, in addition to the consideration of long term benefits to 
300 elephants and other large mammals. 
301 At present, the federal government of Nepal has no mechanism in place to compensate 
302 individuals for crop and property damage due to elephants. Some monies are available for 
303 such damages at the local level. The federal government introduced a compensation policy 
304 for human injury and loss of life from elephants in 2009. Family members of victims of 
305 HEC can receive up to NPR 150,000 (less than US$ 1,600) for loss of life and up to NPR 
306 50,000 (less than US$ 550) for injury.
HEC Mitigation Plans — HEC mitigation plans have been implemented at both the
government and community levels. One approach has been to prevent HEC whereas the
other approach is to limit damage from HEC. Electric fences, walls and ditches have been
constructed to prevent entry of elephants onto croplands and settlements in Nepal and other
Asian countries (Tchamba 1996; Fernando 1997; Sukumar 2003; Choudhury 2004; Shrestha
2007; Lamarque et al. 2009; Pradhan et al. 2011; Gubbi 2012). Along the eastern corridor
connected to India, residents attempt to deter elephants from crossing the border by creating
loud noises such as using firecrackers or drums during the night time (Shrestha 2007).
Relocation of marauding elephants has also been employed to reduce further HEC in some
countries (Fernando 1997; Choudhury 2004), yet not in Nepal to date.
Once elephants have invaded villages and/or cropland, residents have tried to minimize
damage by creating loud noises, or by using fire or fog lights to scare away elephants
(Choudhury 2004; Shrestha 2007). In Africa, noxious sprays have also been used to deter
elephants (Lahm 1994). Generally, these non-lethal approaches have been ineffective as
both Asian and African elephants learn to adapt to these defense systems (Thouless 1994;
Tchamba 1996; Fernando 1997). Poisons have even been used to reduce elephant damage in
India (Choudhury 2004). Some have suggested the culling of elephants as a means of
reducing elephant population sizes and therefore HEC in Africa (Barnes 1996). Seven
elephants were culled by the Nepal government prior to 2000 to reduce HEC (Smith and
Mishra 1992; unpublished data); with the decline of Asian elephant populations throughout
Asia, we consider this a means of last resort to alleviate HEC in Nepal.
Wildlife conservation programs introduced by the government of Nepal have established
protected parks and buffer zones beginning in 1973 for the protection of large mammals and
other threatened wildlife. Subsequently, electric fencing has been installed around those protected park areas in response to HEC and other human-wildlife conflict occurring in the Terai. Fencing is more established in the central and western region of Nepal, which may explain in part the lower HEC in the central and west regions relative to the east. Nonetheless, the fencing around much of the protected parks is in disrepair; further, elephants have been observed knocking down electric fencing to create passages through artificial boundaries in India and Nepal (Choudhury 2004; T. Adhikary, Deputy Director General, Department of National Parks and Wildlife Conservation- Nepal, personal communication) and in India (Choudhury 2004). Several of the elephants killed were by electrocution during the monsoon season when electrical conductivity is most lethal. Additionally, some residents use direct-current fencing, which is more lethal than alternating-current, around their cultivated lands to protect their crops from elephants (D. Neupane, unpublished data).

When electric fencing does not prevent elephant encroachment on croplands and villages, residents may blame conservationists for not keeping elephants confined within the forested areas (Santra et al. 2007), and field conservationists have been physically attacked (Velde 1997; Yadav 2004). In eastern Nepal particularly, where levels of HEC are at their greatest, people’s attitudes are becoming negative towards elephant conservation despite long cultural ties to the elephants (Shrestha 2007; D. Neupane. unpublished data). Scientifically sound and technically feasible management strategies are essential to ultimately reducing HEC (Fernando et al. 2009). Reducing the human imprint by way of strict habitat management and protection is the best way of reducing HEC and ensuring the long-term survival of elephants in the wild (Sukumar and Santiapillai 2006). Options for
reducing that imprint are limited at present due to large increases in human populations, the resultant deforestation and forest degradation in historic elephant ranges (Pradhan et al. 2007; FAO/MoFSC 2009; NPHC 2011), and the lack of financial resources both at the national and local levels (Nagendra et al. 2005).

The lack of adequate corridors between fragmented forests forces the movement of migrating elephants through human settlements to move among forest patches, exacerbating HEC (Sitati et al. 2003; Choudhury 2004; Shrestha 2007). For the present study, the majority of the human and elephant casualties in eastern Nepal occurred in the trans-border corridor of elephants and areas with less forest coverage. This region has the greatest migration of elephants and therefore has the greatest mitigation needs such as electric fencing or other barriers. Further, the re-establishment of forest corridors could reduce human elephant interactions in villages (Choudhury 2004), yet would require the removal of residents at great expense. One example of this type of approach was the relocation of a village located centrally in Chitwan National Park (central Terai), with government subsidies provided for those relocated (Sharma et al. 2011; Dhakal et al. 2011). The establishment of large connected corridors could enhance the geographic scope of HEC and increase damage to surrounding crop lands. From a conservation perspective, an increase in corridors should enhance gene flow and genetic diversity among previously isolated small populations (Schwartz and Mills 2005). More feasible than relocating settlements would be the prevention of further damage to existing corridors and forest preserves; this would not reduce present HEC levels, but also would not exacerbate present levels of conflict which have been on the rise.

As a result of establishing protected reserves, ecotourism associated with large mammals
Human-Elephant Conflict in Nepal

has become a boon to southern Nepal and other Asian countries (Rijal 1997; Santiapillai and Wijeyamohan 2004; Choudhury 2004, Nyaupane and Poudel 2011). Ecotourism dollars flow normally within local economies, improving the socioeconomics of those areas. For example, of the monies generated from national park activities, 30 - 50% is allocated to local developments associated with the buffer zones (Neupane 2007). A more efficient distribution system of ecotourism dollars so as to directly reduce HEC and/or lessen the impact of that HEC could reduce retaliatory killings of Asian elephants. Specific examples of outcomes could lie in seven areas: 1) to enhance the construction and maintenance of electric fencing around areas of highest elephant density (Sukumar 2003; Shrestha 2007); 2) to compensate individuals impacted by elephant damage which could improve people’s attitudes towards elephants and reduce retaliatory killings of elephants (Choudhury 2004; Yadav 2004); 3) to purchase land for establishing corridors and enhance land-use management (Choudhury 2004; Shrestha 2007); 4) to fund research associated with reducing HEC (Choudhury 2004); 5) to train local people to effectively respond to elephant invasion (Yadav 2004); 6) to educate local people on the values of conservation in general and the immediate benefits of ecotourism (Choudhury 2004; Shrestha 2007); and 7) to translocate marauding elephants to protected areas having low elephant density (Yadav 2004). Each practice would require a coordinated government strategy in the redistribution of funds within the country, and represent daunting tasks for a country having few economic resources.

In summary, we have identified an increase in HEC over the past decade as human densities have increased within the Terai of Nepal. Most HEC occurring with crop harvest, particularly rice, and occurred during the nighttime when human activity was lowest. In
addition to our recommendation for preventing further habitat loss and degradation, we have identified several strategies to reduce HEC. While these recommendations will not eliminate HEC, a reduction in HEC should greatly reduce human and elephant mortality.

**ACKNOWLEDGEMENTS**

This work is benefited by funding from the US Fish and Wildlife Service, Arkansas State University, and Mohammad bin Zayad Conservation Fund. We are appreciative of assistance and advice regarding our HEC studies from A. C. Williams of the WWF AREAS. We thank S. Luitel and O. Iseyemi for their assistance in technical and statistical support. We also thank E. Pannkuk and E. Weiss for critically reviewing the manuscript and the anonymous reviewers for improving the quality of this manuscript.

**LITERATURE CITED**


Human-Elephant Conflict in Nepal


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**Table 1.**—Spatial distribution of reported elephant and human casualties in the Terai of Nepal from 2003 – 2012.

<table>
<thead>
<tr>
<th>HEC Region</th>
<th>Human Death</th>
<th>Human Injury</th>
<th>Elephant Death</th>
<th>Elephant Injury</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern</td>
<td>50</td>
<td>30</td>
<td>13</td>
<td>6</td>
<td>99</td>
</tr>
<tr>
<td>Central</td>
<td>41</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>Western</td>
<td>9</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Totals</td>
<td>100</td>
<td>47</td>
<td>16</td>
<td>6</td>
<td>169</td>
</tr>
</tbody>
</table>
Fig. 1a.—Protected areas (PAs), current trans-border elephant migratory routes, and dominant HEC areas of the Terai region of Nepal. Not shown are smaller patchy forests within the Terai.

Fig. 1b.—Frequency of HEC relative to region in Nepal between 2003 – 2012.

Fig. 2.—Frequency of HEC (blue; n = 224) and human casualties (red; n = 147) by year in Nepal from 2003 – 2012.

Fig. 3.—Frequency of HEC relative to crop rotation, season, and by month in Nepal for the years 2002 – 2012. Dates below crops represent growth period to harvest. During April and August, the fields are fallow.

Fig. 4.—Total month-wise frequency of human (red; n = 147) and elephant casualties (blue; n = 22) in Nepal for the years 2003 – 2012.

Fig. 5.—Month-wise frequency of HEC incidents resulting in property damage in Nepal for the years 2003 – 2012. Due to infrequent reporting by the news media these numbers are understated.

Fig. 6.—Age distribution of injured and killed people as a result of elephant attacks in Nepal for the years 2003 – 2012.

Fig. 7.—Frequency of HEC in Nepal relative to time of day for the years 2003 – 2012 (n = 139).
Human-Elephant Conflict in Nepal

![Bar chart showing the number of human-elephant conflict (HEC) cases by month and crop type. The chart includes categories for Spring, Monsoon, and Winter seasons. The months are January, February, March, April, May, June, July, August, September, October, November, and December. The crops are Wheat, Maize, and Rice. The chart indicates a peak in December with a significant number of HEC cases.]
Human-Elephant Conflict in Nepal
Human-Elephant Conflict in Nepal
Human-Elephant Conflict in Nepal

![Bar chart showing the percentage of HEC incidents during different times of the day.](image)

- **Day**: Lowest percentage
- **Evening**: Lower percentage
- **Night**: Highest percentage
- **Morning**: Moderate percentage

**Time of the Incidents**

- Day
- Evening
- Night
- Morning

- **% HEC Incidents**
  - 0% to 80%
PRESENTATIONS
SESSION IX
IN SITU MANAGEMENT OF WILDLIFE 
AND HABITAT
THE SIGNIFICANCE OF PRE-EXISTING SOCIAL BONDS IN TRANSLOCATED BLACK RHINOS

Natasha Anderson (Lowveld Rhino Trust/International Rhino Foundation)
Raoul du Toit (Lowveld Rhino Trust/International Rhino Foundation)
Kevin Dunham

Black rhinos (Diceros bicornis) are generally assumed to be solitary animals in the wild apart from cow-calf pairs and transient mating pairs. This perception may induce some rhino management suggestions that do not adequately take account of social factors. For instance, a standard recommendation in rhino metapopulation management is that, to counter the loss of genetic diversity through genetic drift, at least one new founder should be introduced into a sub-population every generation. This recommendation implies that a rhino can be moved from one population to another without major social problems.

The Lowveld Rhino Trust’s experience of translocation operations suggests that pre-existing social bonds between rhinos are more significant to the success of such operations than has generally been appreciated.

Between 2003 and 2010, 121 black rhinos were introduced into a 2,300 km² section of Bubye Valley Conservancy (BVC), Zimbabwe. These translocations have provided a rare opportunity to observe black rhino behaviour as they involved the phased relocation of entire subpopulations. The translocations were undertaken in response to an expansion, year by year, of human settlement and poaching pressure within Bubiana Conservancy. The rhino populations in both conservancies were monitored at the individual level, and so the home ranges of individual rhinos and the associations between these rhinos both before and after the series of translocations are known. Black rhinos occupy fairly stable home ranges, with dominant bulls overlapping their ranges with those of several cows and sub-dominant animals. Rhinos occupying overlapping or adjacent home ranges can be regarded as neighbours that have fairly regular interactions of one kind or another.

Not all neighbours were translocated from the one conservancy to the other in the same year, because logistical and political constraints limited the scale of each annual operation. Nor were all rhinos released at the same point in BVC. Nonetheless, a clear tendency was shown for released rhinos to re-associate with the same neighbours that they had in Bubiana Conservancy, despite these re-associations requiring some rhinos to move significant distances through unfamiliar territory after release.

The importance of social factors in rhino translocations is also emphasised by observations of rhinos that experienced aggression from other rhinos, or failed to breed initially in BVC, after being brought in from other populations and released as complete strangers at BVC.

The significance of pre-existing social bonds in translocation success is also indicated by the outcome of long-distance translocations of black rhinos from South Africa to North Luangwa National Park in Zambia. Of 25 rhinos released within this area, in several groups, there has been a clear pattern of greater morbidity amongst those that were strangers (i.e. lacked relatives or previous neighbours within that translocated group) compared to those that had prior familiarity with one or more other rhinos within the translocated group.

The conclusion from these translocation experiences is that more tranquil re-introduction scenarios (i.e. less dispersion, less fighting) can be achieved by restocking with rhinos that already know each other prior to their translocation.
THE SIGNIFICANCE OF PRE-EXISTING SOCIAL BONDS IN TRANSLOCATED BLACK RHINOS

NATASHA ANDERSON
Raoul du Toit
Kevin Dunham
LOWVELD REGION OF ZIMBABWE

BUBIANA
125,000 Hectares

BUBYE VALLEY
323,000 Hectares

SAVE VALLEY
354,000 Hectares

KRUGER NATIONAL PARK
1992/3 introductions into Bubiana and Save Valley Conservancies

SUSAN
23/2/M/4

MARIYA
23.02/M/5

2035
MARIYA
2 YEARS OLD
2212 – BRENDA, FIRST CALF OF MARIYA, BORN 1997
2061 - FIONA, SECOND CALF OF MARIYA, BORN 2000
2099 – IRIS, born 2004

1214 – KILO, born 2006
2351 – RUTH, born 2010
2397 – MARIYA’S SIXTH CALF, born 2012
Poaching orphan – “OLIVER” - Susan’s 2008 calf
<table>
<thead>
<tr>
<th>Founders introduced</th>
<th>69</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population end 1993</td>
<td>87</td>
</tr>
<tr>
<td>Calves born from these 1994 -2013</td>
<td>405</td>
</tr>
<tr>
<td>Non-poaching deaths of these</td>
<td>37</td>
</tr>
<tr>
<td>Poaching deaths of these</td>
<td>227</td>
</tr>
<tr>
<td>Population mid 2013</td>
<td>228</td>
</tr>
<tr>
<td>Growth rate realised in 20 years</td>
<td>5%</td>
</tr>
</tbody>
</table>
BUBIANA CONSERVANCY 2002
60,000 out of 125,000 hectares converted to subsistence farming.
Resident population of 110 black rhinos
From 2000-2002:
7 snare incidents
7 poached rhinos (shot or snared)
Southern African average: 5 - 15% of translocated black rhinos die due to post release stress or fighting.

Only $\frac{1}{140} - 0.7\%$ of black rhinos died due to post release stress or fighting in the Bubye Valley introductions.

Why were there so few post release stress or fighting deaths in these translocations?

Large area
Many water points
Low rhino densities
Short translocation distances

MAJORITY OF RHINOS INTRODUCED ALREADY KNEW EACH OTHER
Typical adult cow with calf under two years old.
Typical cow with new born and previous calf.
Cow with her calf and an adult bull
Adult bull, pregnant cow and non-related sub-adult
Adult cow with calf and unrelated sub-adult female
Two unrelated adult cows followed by their respective calves
Map of Bubye showing release sites

- Bubye Valley Conservancy
- SAMMY DIP BOMAS
- CHAMAKUNDAU FREE

Dimensions:
- 2,300km²
- 60km North-South
- 50km East-West
- 10km
SIABUWA (24YR, F) – INTRODUCED 2008

SIX (3YR, F) – INTRODUCED 2008

SINJALO (6YR, M) – INTRODUCED 2008

SIABUWA’S 2009 CALF

SEPTEMBER 2011
IN BUBIANA

IN BUBYE

TRANSLOCATED 2009

GILL

SIX

SIABUWA

SINJALO

LOST

DOZER

ROSEMARY

THREE

MARULA

GANYA

JACKALBERRY
DISTANCE BETWEEN BLACK RHINOS MOVED FROM BUBIANA TO BUBYE 2008 AND 2009

DISTANCE APART IN BUBIANA A (KM) vs. DISTANCE APART IN BUBYE (KM)

- Known neighbours
- Not known
Average black rhino density in Main Section was 1 rhino per 16.6km$^2$.

2009 introduced Ganya - 11 year old bull from Bubiana, moved 14km from release into the area of highest rhino density (1 rhino per 10km$^2$). No indication of any fresh fighting.

2010 introduction Godfrey - 8 year old bull from Save Valley, moved 42km inconsistently from release into an area of very low rhino density (1 rhino per 50km$^2$). Facial lacerations indicate considerable post release fighting.
2009 intro. - Ganya - 11 year old bull

Ganya knew at least 5 other ex-Bubiana rhino in Bubye, found them and settled in.

2010 intro. - Godfrey - 8 year old bull

Godfrey knew no other rhinos and clearly did not settle easily.
### Black rhino reintroductions - North Luangwa, Zambia

<table>
<thead>
<tr>
<th></th>
<th>Number introduced</th>
<th>Satisfactory establishment</th>
<th>Unsatisfactory establishment</th>
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</thead>
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<tr>
<td>Rhinos with some prior familiarity</td>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Rhino with no prior familiarity</td>
<td>18</td>
<td>6</td>
<td>12</td>
</tr>
</tbody>
</table>
Black rhinos maintain strong social bonds that persist through translocations, even if translocations are phased over several years.

A more tranquil reintroduction scenario (i.e. less dispersion, less fighting, quicker breeding) will be achieved by restocking with rhinos that already know each other.
BOMA ADAPTATION AND DEVELOPMENT OF A SCORING SYSTEM FOR RECENTLY CAPTURED WHITE RHINOCEROS (CERATOTHERIUM SIMUM) IN SOUTH AFRICA

Michele Miller, DVM, MPH, PhD
Rare Species Conservatory Foundation
Acknowledgements

- Melandi Kruger
- Marius Kruger
- Ben Baloyi
- Dr. Francisco Olea-Popelka
- Jenny Joubert
- Khosi Mathebula
- Guy Hausler
- Dr. Peter Buss
- South African National Parks
- Colorado State University
- ABAXIS
- International Rhino Foundation
Background

- Risks of white rhino anesthesia and translocation complicated by transport and adaptation to boma

- Mortality estimated to be 5%
  - Morbidity likely to be higher
Study Purpose

- Assess factors influencing individual rhino adaptation to boma conditions
  - Minimize morbidity and possible mortality associated with confinement
Materials and Methods

- 109 white rhinos captured in Kruger National Park, South Africa (2009-2012)
- Immobilized with etorphine, azaperone, hyaluronidase and butorphanol (IV or in dart)
- Received diprenorphine (M50-50) and zuclopethixol acetate (Clopixol-Acuphase) in crates
- Received azaperone and naltrexone prior to release into bomas
Materials and Methods

- Placed in 25 x 50m bomas with vertically spaced wooden poles

>2 weeks
Materials and Methods

- Data collection
  - Immobilization data
    - Drugs
    - Distances run
    - Physiological parameters
    - Blood gases
    - Weight
    - Hematology, biochemistry, vitamin D&E, mineral panels
- Release data
  - Drugs
  - Weight
  - Hematology, biochemistry, vitamin D&E, mineral panels
  - +/- Blood gases
  - +/- Physiological parameters
Materials and Methods

- Scoring system used daily to score adaption
  - Appetite, defecation, demeanour, activity
  - Scored 1-5 for healthy rhinos
  - Scores for ill rhinos (-1, -3, -5)
- One of two boma managers scored rhinos daily until released
<table>
<thead>
<tr>
<th>Score</th>
<th>Appetite</th>
<th>Defaecation</th>
<th>Demeanour/Behaviour</th>
<th>Activity levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Eating 90% to 100% of normal intake</td>
<td>Brownish / green large stool (multiple defaecations per day)</td>
<td>Very calm</td>
<td>Standing stationary or sleeping and / or ears towards stimulus*</td>
</tr>
<tr>
<td>4</td>
<td>Eating 50% to 75% of normal intake</td>
<td>Dark brownish / green medium stool (3 to 5 balls more than once a day)</td>
<td>Calm and not nervous</td>
<td>Walks away slowly or stands their ground in response to stimulus*</td>
</tr>
<tr>
<td>3</td>
<td>Eating 25% to 50% of normal intake</td>
<td>Dark small stool (1 or 2 balls more than once a day)</td>
<td>Mildly nervous and / or aggressive</td>
<td>Trots and / or walks for a short distance in response to stimulus*</td>
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<tr>
<td>2</td>
<td>Eating 0% to 25% of normal intake</td>
<td>Putty-like dark, small stool or loose faeces</td>
<td>Moderately nervous and / or aggressive</td>
<td>Runs or trots away and charges once or twice in response to stimulus*</td>
</tr>
<tr>
<td>1</td>
<td>Not eating at all</td>
<td>Not defaecating</td>
<td>Extremely nervous and / or aggressive</td>
<td>Runs around and / or frequently charges and / or hits poles / doors in response to stimulus*</td>
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<tr>
<td>-1</td>
<td>-</td>
<td>Stool is loosely formed (similar to domestic cow)</td>
<td>Mildly depressed</td>
<td>Walks a short distance in response to stimulus*</td>
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<tr>
<td>-3</td>
<td>-</td>
<td>Diarrhoea (light brown or green in colour)</td>
<td>Moderately depressed</td>
<td>Standing stationary or sleeping and turns head and / or ears towards stimulus*</td>
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<tr>
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<td>Profuse watery diarrhoea (dark brown / black in colour)</td>
<td>Very depressed</td>
<td>Recumbent with no response to stimulus*</td>
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Individual Median Rhino Boma Scores 2011

The diagram shows the median scores for individual rhinoceroses. The x-axis represents the individual rhinoceros number, while the y-axis represents the median score. The scores range from 0 to 20, with a median score of 10 indicated by a horizontal line.
Median Daily Boma Scores for White Rhino by Year

- 2009 MEDIAN
- 2010 MEDIAN
- 2011 MEDIAN
Individual Rhino Boma Score by Day (First 21 Days) 2011
Distribution (25th percentile, median and 75th percentile) for Total Score Each Day Among Rhinos That Adapted and Those That Did Not Adapted to Boma Conditions  2009-2011

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<td>86</td>
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| Wilcoxon Rank sum test a | p-value | 0.706 | 0.3835 | 0.2205 | 0.8946 | 0.9055 | 0.2774 | 0.0688 | 0.0012 | 0.0003 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.003 |

a The Wilcoxon Rank sum test compares the median score value between adapted and maladapted rhinoceroses
## Distribution of Adapted/Maladapted Rhinos

<table>
<thead>
<tr>
<th></th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Adapted rhinos</td>
<td>35</td>
<td>25</td>
<td>13</td>
<td>9</td>
<td>82</td>
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<tr>
<td>Rhinos with complications not requiring release*</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>6</td>
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<tr>
<td>Maladapted rhinos – released/relocated</td>
<td>11</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>21(^a)</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>28</td>
<td>18</td>
<td>13</td>
<td>109</td>
</tr>
</tbody>
</table>

\(^a\)19.3% maladapted (95% CI 12.3\%-27.9%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>n</th>
<th>P-Value</th>
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<tbody>
<tr>
<td><strong>Ataxia</strong></td>
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<tr>
<td>(minutes)</td>
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<td>2.36</td>
<td>3.22</td>
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<td>3.35</td>
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<td>2.28</td>
<td>4.4</td>
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<tr>
<td><strong>Down Time</strong></td>
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<tr>
<td>(minutes)</td>
<td></td>
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<td>5.63</td>
<td>6.82</td>
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<td><strong>Crate Time</strong></td>
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<tr>
<td>(minutes)</td>
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<td><strong>Dist. Before Dart</strong></td>
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<tr>
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<td>590.8</td>
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<td><strong>Dist. After Dart</strong></td>
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* P-Value was acquired using T-tests comparing each parameter between adapted and maladapted rhinos at time of capture** (1st sample)
<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
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<td>9.87</td>
<td>12.70</td>
<td>63</td>
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<tr>
<td>Maladaptive</td>
<td>9.04</td>
<td>6.24</td>
<td>4.58</td>
<td>13.50</td>
<td>10</td>
</tr>
</tbody>
</table>

*P-Value was acquired using T-tests comparing each parameter between adapted and maladapted rhinos at time of capture (1st sample)
Comparison of Adapted/Maladapted Rhinos at Time of Capture

- No differences in age, sex, body weight
- No differences in hematologic parameters
  - Wbc, hct, differential counts
- No differences in biochemical parameters EXCEPT
  - BUN (11.1 vs 9.1 mg/dl)
  - AST (68.9 vs 52.8 U/L)
  - Adapted vs maladapted (p<0.05)
Changes During Boma Confinement

Hematology of Adapted Rhinos at Capture and Release

<table>
<thead>
<tr>
<th></th>
<th>Capture</th>
<th>Release</th>
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<tbody>
<tr>
<td>WBC*</td>
<td>16.9</td>
<td>14.9</td>
</tr>
<tr>
<td>Neutr %*</td>
<td>43.1</td>
<td>51.2</td>
</tr>
<tr>
<td>Lymph %</td>
<td>25.5</td>
<td>24.9</td>
</tr>
<tr>
<td>Mono %</td>
<td>13</td>
<td>14.7</td>
</tr>
<tr>
<td>Eos %*</td>
<td>16.2</td>
<td>8.9</td>
</tr>
<tr>
<td>Baso %*</td>
<td>1</td>
<td>0.03</td>
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<tr>
<td>Bands %</td>
<td>0.4</td>
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<tr>
<td>Hct %</td>
<td>44.3</td>
<td>47.4</td>
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</table>
Changes During Boma Confinement

<table>
<thead>
<tr>
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<tr>
<td>ALP U/L*</td>
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<td>AST U/L*</td>
<td>68.9</td>
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<tr>
<td>CK U/L*</td>
<td>200.8</td>
<td>12.8</td>
</tr>
<tr>
<td>GGT U/L*</td>
<td>56.1</td>
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<tr>
<td>Mg mg/dl*</td>
<td>68.9</td>
<td>10.4</td>
</tr>
<tr>
<td>BUN mg/dl*</td>
<td>200.8</td>
<td>18.7</td>
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<tr>
<td>Alb g/dl*</td>
<td>200.8</td>
<td>11</td>
</tr>
<tr>
<td>TP g/dl*</td>
<td>56.1</td>
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<tr>
<td>Glob g/dl</td>
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<tr>
<td>Ca mg/dl</td>
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</tr>
<tr>
<td>P mg/dl</td>
<td>56.1</td>
<td>9.8</td>
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</table>

* denotes significant difference.
# Changes During Boma Confinement

Hematology of Maladapted Rhinos at Capture and Release

<table>
<thead>
<tr>
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<th>WBC</th>
<th>Neutr %*</th>
<th>Lymph %*</th>
<th>Mono %</th>
<th>Eos %*</th>
<th>Baso %</th>
<th>Bands %*</th>
<th>Hct %</th>
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</thead>
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<td>16.4</td>
<td>42.3</td>
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<td>14.8</td>
<td>0.8</td>
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<tr>
<td>Release</td>
<td>22.6</td>
<td>54.2</td>
<td>19.5</td>
<td>12.4</td>
<td>5.1</td>
<td>0</td>
<td>4.4</td>
<td>37.2</td>
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Changes During Boma Confinement

<table>
<thead>
<tr>
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<th>CK U/L</th>
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<td>181.6</td>
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<tr>
<td>Release</td>
<td>74.2</td>
<td>65.3</td>
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<table>
<thead>
<tr>
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<th>GGT U/L*</th>
<th>Mg mg/dl</th>
<th>BUN mg/dl</th>
<th>Alb g/dl</th>
<th>TP g/dl</th>
<th>Glob g/dl</th>
<th>Ca mg/dl</th>
<th>P mg/dl</th>
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<tr>
<td>Capture</td>
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<td>3.06</td>
<td>9.1</td>
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<td>7.1</td>
<td>12.1</td>
<td>4.3</td>
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<tr>
<td>Release</td>
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<td>2.31</td>
<td>17.4</td>
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<td>10</td>
<td>7.1</td>
<td>11.6</td>
<td>3.8</td>
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### Physiological Values at Release for Maladaptive vs Adaptive

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adaptive</th>
<th>Maladaptive</th>
<th>Mean</th>
<th>SD</th>
<th>p25</th>
<th>Median</th>
<th>p75</th>
<th>N</th>
<th>P-Value</th>
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<tr>
<td>WBC</td>
<td></td>
<td></td>
<td>14.89</td>
<td>4.28</td>
<td>12</td>
<td>14.2</td>
<td>18.4</td>
<td>63</td>
<td>0.0006</td>
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<tr>
<td>Lymph (%)</td>
<td></td>
<td></td>
<td>24.94</td>
<td>9.07</td>
<td>19</td>
<td>25</td>
<td>31</td>
<td>63</td>
<td>0.0458</td>
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<tr>
<td>Eos (%)</td>
<td></td>
<td></td>
<td>8.92</td>
<td>5.06</td>
<td>4</td>
<td>9</td>
<td>12</td>
<td>63</td>
<td>0.0088</td>
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<tr>
<td>Bands (%)</td>
<td></td>
<td></td>
<td>0.13</td>
<td>0.61</td>
<td>0</td>
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<td>0</td>
<td>63</td>
<td>&lt;0.0001</td>
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<tr>
<td>Hct (%)</td>
<td></td>
<td></td>
<td>47.4</td>
<td>5.3</td>
<td>47.6</td>
<td>14</td>
<td></td>
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<td>&lt;0.0001</td>
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<tr>
<td>Ca mg/dl</td>
<td></td>
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<td>12.19</td>
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<td>Fe ppm</td>
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<td>2.55</td>
<td>1.04</td>
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<td>190</td>
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<td>344</td>
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<td>PHOS mg/dL</td>
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<td>4.8</td>
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<td>73</td>
<td>&lt;0.0001</td>
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<tr>
<td>MG mg/dl</td>
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<td>2.77</td>
<td>0.38</td>
<td>2.5</td>
<td>2.8</td>
<td>3</td>
<td>73</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*P-Value was acquired using T-tests comparing each parameter at release by adaptation group.*
Results

- 4 year period 2009-2012
  - 21/109 captured rhinos maladapted (19.3%)
  - Additional 5.5% with minor complications
  - 1 mortality (suspected acute Salmonellosis)

- Score of 16/20 = successful boma adaptation
  - Median period for 2009-2011 period – 19 days
  - Consistent with managers’ observations of 3-4 weeks
  - Similar to recommendations for boma adaptation for black rhinos
Results

- Mean length of boma confinement
  - Adapted rhinos - 89.9 days (range 39-187 days)
  - Maladapted rhinos - 13 days (range of 8-16 days)
- No significant differences in the gender, age, or weight of rhinos initially placed in the bomas
- Capture-related and physiological measurements did not correlate with rhino boma adaptation status
- No significant differences in hematologic, biochemical, mineral and vitamin panels at the time of capture between adapted and maladapted rhinos
Results

- Boma-adapted rhinos had minor hematological and biochemical changes between capture and release suggestive of mild-moderate negative energy balance (increased CK and BUN).

- Mean weight loss was 65.9 kgs (SD 77.8 kgs) while in the bomas.

- Vitamin D (92.0 to 78.8 ng/ml) and E (2.70 to 1.46 ng/ml) values were significantly lower at release compared to capture.
Results

- Boma-maladapted rhinos showed significant stress hemograms (22,600 white blood cells with 4.4% bands) at the time of release despite the short time in confinement.

- Decreased mean hematocrit (55 to 37.2%) and drop in mean iron (3.01 to 0.97 ppm) indicated possible blood loss through gastrointestinal ulceration.

- Biochemical values suggested significant catabolic states due to negative energy balance.
  - Consistent with the larger mean weight loss (224.0 +/- 61.1 kgs) – approximately 14.7% of body weight.
Conclusions

- White rhinos appear to take an average of approximately 3 weeks to adapt to boma confinement (minimum)

- Significant difference between maladapted and adapted rhinos were detected starting at day 8

- Second week in boma critical monitoring period
Conclusions

- Individuals that do not show a steady increase in scores over the first 7-10 days should be considered release candidates
Conclusions

- The scoring system for boma adaptation could be modified for other rhino species and systems, minimizing subjectivity in assessing rhino relocation management.

Ziwa Rhino Sanctuary, Uganda
CONTACT CALLS OF THE NORTHERN AND SOUTHERN WHITE RHINOCEROS: SOURCE OF INFORMATION ON INDIVIDUAL IDENTITY AND SPECIES OF THE CALLER?

Ivana Cinková,1* Richard Policht²,³

¹Department of Zoology and Lab of Ornithology, Faculty of Science, Palacký University, 17. listopadu 50, 771 46 Olomouc, Czech Republic, ivanacinkova@centrum.cz; ²Department of Ethology, Institute of Animal Science, Prague, Czech Republic; ³Department of Game Management and Wildlife Biology, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Czech Republic

Communication of the northern (Ceratotherium cottoni) and southern white rhinoceros (Ceratotherium simum) has, until now, been studied very little. The rhinos have poor eyesight and vocal and olfactory signals are the most important for their communication. The vocal repertoire of some rhinoceros species has recently been described, however, information the rhinos are able to transmit and perceive through their vocalizations remains unknown as studies reporting any information encoded in rhino calls are completely missing. White rhinos are the most social of all rhinoceros species and a well-developed communication system might therefore be especially useful to them. We studied the contact call ‘pant’ of the northern and southern white rhinos, which is formed by a series of inhalations and exhalations and is somewhat unique to the white rhinoceros. We investigated if pant calls contain information on individual identity, species and sex-age class of the caller. Such ability, in addition to olfactory cues, would allow rhinos to communicate with highly increased accuracy.

We recorded and analysed pant calls of northern and southern white rhinos in several zoological gardens and South African wildlife reserves and also conducted playback experiments with pant calls on wild southern white rhino bulls in South Africa. We analysed 385 pant calls of six northern and 14 southern white rhinos for individuality and species differences. Discriminate analysis assigned a high percentage of pant calls to the correct animal. Calls of individuals clustered into apparently separate groups according to the species, and species differed significantly in call duration and in several frequency parameters of their calls. We also tested pant calls of 33 southern white rhinos for differences among the sex-age classes (females, sub-adult males, adult males in visual isolation from other rhinos, adult males when approaching a female) and a discriminant analysis classified the calls with a high accuracy. Playback experiments with pant calls were conducted on nine wild territorial bulls and we investigated if they react differently to the pant calls of females and adult territorial males. The bulls reacted significantly more intensively to the female than male contact calls. The bulls
also spent more time walking and running and they showed a shorter latency to mark the territory after the playback of female compared to male pant calls.

White rhino pant calls have complex structure and can also potentially encode other information. Therefore, they might represent a more sophisticated communication system than what is currently known in rhinos. Better knowledge of vocal communication of northern and southern white rhinos and how it stimulates social and reproductive behaviour might be extremely valuable for improving their management in zoological gardens and wildlife reserves.
Human - Elephant Conflict in the North West Wildlife Zone

Sri Lanka

By
Pubudu Weerarathna
Chairman

Species Conservation Center
The Conflict

Conflicts between humans and animals are a serious problem in Sri Lanka. The damage and destruction of natural habitats by humans is the main cause. Poses a danger for the community and to their properties. With the animals often killed, captured or harmed, these conflicts are one of the main threats to the continued survival of many species.
Human Deaths

(Approximately)
Sources: Epidemiology Unit, Public Health Veterinary Services

- Mosquitoes – 186
- Snakes – 100
- Rats – 96
- Elephants – 52
- Dogs – 41
- Crocodiles – 5
The Human-Elephant Conflict in Sri Lanka
Number of elephant deaths

Regional Distribution of Elephant Deaths (2005 to 2012)
Human - Elephant Conflict in the North West Wildlife Zone

• The North Western Province comprising of two administrative districts, Puttalam (3,013 km²) and Kurunegala (4,813 km²) supports nearly 20% (1189 elephants) of the estimated elephant population of Sri Lanka (DWC, 2011).
Statement of the Problem

• The DWC has divided the county to six regions for management purposes. The North Western region is the smallest region among the six. The north western region comprise of two districts, Puttlam and Kurunagala. The province has an extent of 7,888 km² and an estimated human population of 2,184,136 (Department of Census and Statistics, 2005).

• The north western region has 46 divisional secretariats of which 16 are affected by human elephant conflict.
Number of elephant deaths that have taken place in the
Northwestern Region

Elephant Deaths in North Western Region (2005-2012) (Department of Wildlife, Sri Lanka)
Research Objectives

• This study has been carried out with the assumption that north western province communities are highly effected by human elephant conflict, their social interactions with other human beings and the challenges offered by their livelihoods. Therefore, the objective is to identify accelerating causer for the HEC and potential solutions (medium and short-term) for HEC through community participation in collaboration with the DWC.
The secondary research objectives are

- To identify the areas with escalating human elephant conflict in the Kurunagala and Puttlam Districts
- To identify isolated forest areas used as refugees by the elephant population
- To document the activities carried out by both government and non-government organizations, who are engaged in providing solutions to this problem
- To document how local organizations/communities cope with the problem
Research Methodology

• The conflict data was gathered from the Department of Wildlife Conservation (DWC) and the office of the Divisional Secretary (DS).

• Based on the data obtained on conflict sample villages were identified and a socio-economic survey was conducted by visiting these villages with Grama Niladari (GN) (Village administrative officer).

• The location of electric fences, isolated forest areas, forest boundaries and traditional elephant corridors were mapped based on data collected using a GPS (Global Positioning System).
Crop Damage

• Just like there are extensive crop damages, property damages are equally high. Approximately 67% of the total GNDs covered in Puttalam, 65% in Kurunegala
Socio Economic Issues

• According to the available statistics from the 14 selected divisional secretariats in the 2 Districts, more than 50% are engaged in agriculture as the main livelihoods. In the Puttalam district nearly 20% depends on agriculture and the rest depend on the fishery and salt industry. Whereas in the Kurunagala district 35% of the population depends on agriculture and forestry industry and the human-elephant conflict directly affects them.
Percentage of Agriculture and Forestry Industry employed population by district
Elephant Corridor/Jungle Corridor

Extent of designated areas administered by Forest Department and Department of Wildlife Conservation (Source: IUCN&WCMC 1997)
Loss of habitats and Habitat fragmentation
Cattle and Buffalo Population

Buffaloe Population

- Kurunegala
- Puttalam

Legend

- Districts:
  - ANURADHAPURA
  - BATTICALOYA
  - HAMBANTOTA
  - MAHANR
  - MONAVALA
  - POLONNARUWA
  - PUTTALAM
  - TRINCOMALEE

Map showing districts color-coded with a key to the left.
Electric Fence

• To minimize the human-elephant conflict DWC has installed more than 1200 km of electric fences along the areas that are managed by the department. After installing an electric fence, the villagers and the community based organizations are responsible for the maintenance of the fences.
Administrative Boundary’s

Authority

• CEA Central Environmental Authority
• DFAR Department of Fisheries and Aquatic Resources
• DWLC Department of Wildlife and Conservation
• FD Forest Department
• SLRDC Sri Lanka Land Reclamation and Development Corporation
Dumping of Garbage

- Interest on different tastes
- Fear is lost between humans & elephants
- Rising of diseases
Locally made explosive devise
A Victim of Land Mines,
Silavathura, Mannar
Damages to Humans & Their Properties & Crops
Lunugamwehera National Park Illegal Forest Clearance in the Buffer Zone,
Illegal Clearance of the elephant corridor in Handapanagala
Clearing of forest along the Kitulkote Proposed elephant corridor
Train Accident
Main Railway Lines in Sri Lanka
Train Accident in Punawa, Madawachchi
Man made agricultural well
Solutions

Immediate

Long Term
Short Term

• Rescue & Rehabilitation

Why????

 Authorities acting immediately before the communities take action
Long Term

• Habitats Enrichment
• Electric Fence
• Identifying isolated forest areas and declare as a forest reservation
• GPS data collecting
• Re-creating of Jungle Corridors
Acknowledgments

• Embassy of the United States of America – Sri Lanka
• Staff of Species Conservation Center
Thank You
Oral imipramine and intravenous xylazine for pharmacologically-induced ex copula ejaculation in an African elephant (*Loxodonta africana*)

Ray L Ball, DVM and Chris Massaro, Tampa’s Lowry Park Zoo
Freezing African Elephant Semen as a New Population Management Tool

Robert Hermes\textsuperscript{1*}, Joseph Saragusty\textsuperscript{1}, Frank Göritz\textsuperscript{1}, Paul Bartels\textsuperscript{2}, Romain Potier\textsuperscript{3}, Barbara Baker\textsuperscript{4}, W. Jürgen Streich\textsuperscript{1}, Thomas B. Hildebrandt\textsuperscript{1}

\textsuperscript{1} Department of Reproduction Management, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany, \textsuperscript{2} NZG Biobank, National Zoological Gardens of South Africa, National Research Foundation, Pretoria, Republic of South Africa, \textsuperscript{3} ZooParc de Beauval, Saint Aignan sur Cher, France, \textsuperscript{4} Pittsburgh Zoo and PPG Aquarium, Pittsburgh, Pennsylvania, United States of America
Semen collection

- passive semen collection technique yields only stored sperm in a very low volume of seminal plasma
Improvement on manual collection?

- Repeatable
- Without anesthesia
- Potentially dangerous animal
- Labor/training effort
- Fresh ejaculate

- Pharmacologically-induced ex copula ejaculation in equids
Pharmacologically-induced ex copula ejaculation in equids

• stallions with limb weakness or pain that precludes safe mounting or ground collection
• injury of the penis
• inadequate libido
• failure of unknown etiology
Pharmacology of ejaculation

• Erection, emission, and ejaculation
  – sympathetic nervous system
  – alpha-adrenergic stimulation and smooth muscle contraction.

• Emission is thought to be primarily an a1 event

• Ejaculation being primarily mediated through a2 stimulation
Pharmacologically-induced ex copula ejaculation in equids

• Oral or intravenous imipramine

• Alpha-2 agonist
  – Detomidine or xylazine

• prostaglandin F2a (PGF2a)
  – 0.01–0.15 mg/kg
  – smooth muscle stimulation

• Combinations of oral or intravenous imipramine with intravenous alpha-2 agonist.
Pharmacologically-induced ex copula ejaculation in equids

Table 1
Summary of pharmacologically-induced-ejaculation regimens for stallion and rates of success

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Rate of ejaculation (number of stallions)</th>
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<tbody>
<tr>
<td>Xylazine</td>
<td></td>
</tr>
<tr>
<td>McDonnell and Love, 1991 (laboratory study)</td>
<td></td>
</tr>
<tr>
<td>Detomidine Rowley et al., 1999 (laboratory study)</td>
<td></td>
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<tr>
<td>Imipramine McDonnell and Turner, 1994 (laboratory study)</td>
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</tr>
<tr>
<td>Imipramine followed by Xylazine McDonnell and Odian, 1994 (laboratory study)</td>
<td></td>
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<tr>
<td>McDonnell and Turner, 1994 (laboratory study)</td>
<td></td>
</tr>
<tr>
<td>Johnston and DeLuca, 1998 (farm practice)</td>
<td></td>
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<tr>
<td>Prostaglandin F2α McDonnell 1992 (laboratory study)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27% (n = 28)</td>
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<td></td>
<td>50% (n = 1)</td>
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<td>42% (n = 5)</td>
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<td>33% (n = 8)</td>
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<td>53% (n = 5)</td>
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<td></td>
<td>57% (n = 6)</td>
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<tr>
<td></td>
<td>75% (n = 8)</td>
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</table>
Imipramine

• tricyclic antidepressant
  – inhibits reuptake of several neurotransmitters, including dopamine, norepinephrine, and serotonin
  – alpha-adrenergic stimulation
• used in human for treatment of
  – aspermia
  – premature ejaculation
  – retrograde ejaculation
Xylazine

• Alpha 2 agonist sedative and muscle relaxant
• reversible
• affect contractions of genital smooth muscle in the horse
  – and had been associated with the occasional side effect of induced-ejaculation
  – Xylazine alone will induce ejaculation ~27% of treated horses
Imipramine and Xylazine

• xylazine alone typically has volume, sperm concentration, pH and total sperm number similar to those of ejaculates obtained in copula

• Treatment with imipramine appears to enhance contraction of the ampulla, and inhibit contraction of the accessory sex glands
  – with the resulting ejaculates of lower volume, higher sperm concentration, higher total number of sperm, and lower pH than for in copula ejaculates
Imipramine and Xylazine

• imipramine at 3mg/kg orally
• two hours later xylazine 0.66mg/kg intravenously
• Ejaculate produced:
  – 1–3 min after xylazine
  – 15 and 25 min after arousing from sedation
• titration of dose to individual stallions significantly improves the rate of ejaculation for any given treatment
Imipramine and Xylazine

• rate of successful ejaculation is affected by the level of arousal (calm or excited) of the stallion at the time of treatment
  – with higher success in quiet and undisturbed stallions
• Teasing stallions before imipramine also reported useful
Imipramine/Xylazine in African elephant

- 23 year old
- 4223kg
Trial dosing

• 12500mg imipramine (2.95mg/kg) po
• followed by 100mg xylazine (0.024mg/kg) IV two hours later.
• very light tranquilization with his penis protruded
  – 20 minutes the elephant moved out of the chute and recovered uneventfully.
  – Later a few drops of fluid were reported to be seen dripping from his penis but these were not recovered.
Additional Trials

• 4 trials over 6 days
• Imipramine ranging from 12500 to 20500 (2.95 – 4.85mg/kg)
• 170mg (0.04mg/kg) xylazine two hours later IV
• Held in ERD 20-30 minutes
• Moved out and monitored
• Each trial he head pressed and bellowed
  – Ejaculation??
Results

• Varied
  – Few drops with sperm to small AI dose
• Best results were ~30 minutes after recovery
• Light rectal massage
• 4ml of thick sperm rich coagulum
  – Diluted with Hepes for evaluation
  – Diluted to 12ml for AI attempt
TLPZ Experience

• Exhausted bull
  – Horse attempts every 3 days
  – TLPZ: 4 attempts in 6 days

• Teasing
  – Best sample was when female had just ovulated

• Timing
  – 30 minutes after recovery from sedation

• Titrating dose
Ejaculation

• Head pressing and bellowing
  – Behaviorally suggestive of ejaculation
  – Sample lost in long repro tract?
  – US exam of ampulla subjectively shows size change
Next steps

• Prep bull
  • Teasing
  • Rectal massage before
    – Timing of collection post-xylazine
• Move out
• Second rectal massage
  – Catheterize while sedated after ejaculation?
  – More complete US exams
Acknowledgements

TLPZ Elephant Team
TLPZ Veterinary Staff
Dr. Trevor Gerlach
Dr. Nico Maldonado
Sperm Cryopreservation and Seminal Plasma Analyses in Asian Elephants (*Elephas maximus*)

**Authors**
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This presentation summarized key note findings from two recent manuscripts:


Asian elephant spermatozoa are sensitive to chilling and do not respond well to cryopreservation. The objectives of the present study were to: (1) determine whether cholesterol content can be modified by preincubation of Asian elephant spermatozoa with cholesterol-loaded cyclodextrin (CLC); and (2) assess the effects of CLC concentration(s), temperature at time of glycerol addition (22°C vs 4°C) and dilution medium on post-thaw sperm survival. Spermatozoa incubated with ≥1.5 mg CLC exhibited increased (P < 0.05) cholesterol concentrations. Pretreatment of spermatozoa with 1.5 mg CLC resulted in improvements (P < 0.05) in all post-thaw parameters. Glycerol addition at 4°C also improved all post-thaw parameters compared with 22°C. Dilution of thawed spermatozoa in an egg yolk-based medium improved (P < 0.05) motility compared with Ham’s F-10 culture medium. In summary, our findings indicate that modifying cholesterol content within the plasma membrane improves the cryosurvival of Asian elephant spermatozoa. The development of an improved cryopreservation method that includes modification of membrane cholesterol and the addition of glycerol at 4°C, as reported in the present study, is an important step towards utilization of cryopreserved spermatozoa in captive management of this species. As a result of this study, a Genome Resource Bank has been established for Asian elephants at the Ringling Bros. Center for Elephant Conservation.


Asian elephants (*Elephas maximus*) have highly variable ejaculate quality within individuals, greatly reducing the efficacy of artificial insemination and making it difficult to devise a sperm cryopreservation protocol for this endangered species. Because seminal plasma influences sperm function and physiology, including sperm motility, the objectives of this study were to characterize the chemistry and protein...
profiles of Asian elephant seminal plasma and to determine the relationships between seminal plasma components and semen quality. Ejaculates exhibiting good sperm motility (≥ 65%) expressed higher percentages of spermatozoa with normal morphology (80.3 ± 13.0 vs. 44.9 ± 30.8%) and positive Spermac staining (51.9 ± 14.5 vs. 7.5 ± 14.4%), in addition to higher total volume (135.1 ± 89.6 vs. 88.8 ± 73.1 ml) and lower sperm concentration (473.0 ± 511.2 vs. 1313.8 ± 764.7 x 10⁶ cells ml⁻¹) compared to ejaculates exhibiting poor sperm motility (≤ 10%; P < 0.05). Comparison of seminal plasma from ejaculates with good versus poor sperm motility revealed significant differences in concentrations of creatine phosphokinase, alanine aminotransferase, phosphorus, sodium, chloride, magnesium, and glucose. These observations suggest seminal plasma influences semen quality in elephants. One- and two-dimensional (2D) gel electrophoresis revealed largely similar compositional profiles of seminal plasma proteins between good and poor motility ejaculates. However, a protein of ~80 kDa was abundant in 85% of ejaculates with good motility, and was absent in 90% of poor motility ejaculates (P < 0.05). We used mass spectrometry to identify this protein as lactotransferrin, and immunoblot analysis to confirm this identification. Together, these findings lay a functional foundation for understanding the contributions of seminal plasma in the regulation of Asian elephant sperm motility, and for improving semen collection and storage in this endangered species.
PRESENTATIONS
SESSION XI
EX SITU REPRODUCTION AND MANAGEMENT
Once spread across the entire northern part of the Indian subcontinent, the greater one-horned rhinoceros is currently considered vulnerable (Talukdar et al, 2008). Given this species is conservation dependent, it is vital to have sustainable populations within zoological facilities. This requires putting significant time and resources into the greater one-horned rhino population and determining factors leading to high reproductive success. In spring 2012 the San Diego Zoo Safari Park experienced a difference between two greater one-horned rhinoceros births. One was a successful live birth, anterior presentation, and the other was a dystocia stillbirth, posterior presentation. Initially the stillbirth was suspected to be due to the posterior presentation at birth. However, years of anecdotal evidence suggested otherwise and a formal investigation was initiated.

Data were gathered by reviewing animal records including behavioral and breeding records for all three species of the San Diego Zoo Safari Park’s rhinoceros. Data on 173 rhino births between 1970 and 2013 were analyzed for species of rhino, and whether or not the birth was recorded as a live birth or stillbirth. Breeding records indicated that 5.3% (n = 93 total births) of the southern white rhinos born at the park were stillborn compared to 0.6% for black rhinos (n = 15 total births) and 24.5% for greater one-horned rhinos (n = 65 total births). Compared to the studbooks for each of these species through to 2010, records indicate southern white rhinos have a captive born stillborn rate of 7%, black rhinos at 11.0%, and greater one-horned rhinos at 19.7%. This information led to further investigation as to why greater one-horned rhinos have such a high rate of stillborns.

The North American Studbook for greater one-horned rhinos lists 126 births at 35 institutions of which 29 were stillbirths. The San Diego Zoo Safari Park alone represents approximately 51.6% of births for greater one-horned rhinos within zoological institutions. Similarly, 16 of the 29 stillbirths have occurred at the park representing 55.2% suggesting the park is quite representative of the greater one-horned rhino population within zoological institutions and this high percentage of still births is not an abnormal occurrence for just one institution. Thus, determining the cause behind the higher percentage of stillbirths could substantially help this population increase sustainability.

While data are limited, 12 births have been video recorded at the San Diego Zoo Safari Park including 8 greater one-horned, 2 southern white and 2 black rhinos. The vast majority of births at the park happen when no one is around and/or no data relevant to this study could be recorded.
Information from these videos was gathered on delivery presentation and presences of sac rupture. Seven additional births had written documentation for presentation and are included but are not considered for sac rupture analysis. Three of these were also recorded, but the video is either lost or misplaced and is not available to confirm sac rupture. Delivery presentation was analyzed as follows. If a calf was born with its front feet and head presenting first it was considered an anterior presentation. If a calf presented with its rear feet first it was considered a posterior presentation. Additionally feet positioning is crucial for presentation and normal delivery. The feet need to be hoof pad “down” for an anterior delivery, as opposed to hoof pad “up” for a posterior delivery.

Of the 19 births 11 calves were delivered in posterior presentation and 7 were delivered in anterior presentation, and one is unknown. Eight of the 12 births that were recorded were live births, 4 were stillborns. In all four cases of a stillbirth delivery, the amniotic sac ruptured prior to delivery of the stillborn. Three were posterior presentation, and one is unconfirmed. Additionally all greater one-horned live births recorded did not have the sac rupture prior to delivery. However, one black rhino birth had the sac rupture prior to birth but still resulted in a live birth, so the number of minutes between sac rupture and delivery may be significant.

Looking at position, one stillborn in this study presented initially with a posterior presentation, but the pads of the feet were facing downward. Since the calf was delivering back feet first, this was an “upside down” position in the birth canal. This female over the course of several hours had the calf rotate to the proper position before delivery but resulted in a stillborn. While anterior versus posterior delivery may not be significant, the position of the calf with pads up or down may be significant, further information is still needed.

With the limited results and surrounding questions, rhino births published on Youtube were then incorporated into the data. The caveat for addition of these recordings is that all births are live births, as no facility would be expected to post video of a stillbirth. Thus these recordings do not necessarily add to the evidence for premature sac rupture as evidence for a stillborn. Nine Youtube videos of rhinos giving birth were evaluated including 4 southern white rhinos, 2 black rhino and 3 greater one-horned rhinos. Data from the southern white rhinos shows that 3 delivered in anterior presentation, 1 was in the posterior presentation. Both black rhino deliveries were anterior presentation and the 3 greater one-horned rhinos included 1 anterior and 2 posterior. All nine rhinos had the amniotic sac intact at time of delivery.

During conversations at the San Diego Rhino Keeper Workshop in May of 2013, additional video data was offered by the Chester Zoo, England. In 2012 they had 2 black rhino give birth; one was a dystocia, posterior presentation with amniotic sac rupture that resulted in the assisted delivery of a dead calf. This calf was delivered over 24 hours after the initial presentation of a single back foot. The other was a normal anterior birth, sac intact at time of delivery.
Additional data was also submitted by the Buffalo Zoo and Cincinnati Zoo and Botanical Gardens. All of the submitted data was recorded.

Preliminary data suggests that presentation at delivery must not be discounted as a factor in stillbirths in rhinoceros considering all 4 stillbirths were posterior presentation. The single live birth with premature sac rupture (a black rhino) indicates that even if the sac ruptures, a live birth is possible. The link may be the time between sac rupture and delivery of the calf. One theory is that the amniotic sac needs fluid in it to help protect the umbilical cord from compression during delivery. If the sac is ruptured and the cushioning effect of the fluid is lost, pressure on the umbilicus may result in loss of oxygen to the calf. Therefore premature sac rupture must be considered as a possible link to stillborn.

Rhinoceros birthing has been observed dozens of times in zoos with a general consensus on what constitutes a “normal” birth. A more comprehensive evaluation of what constitutes a “normal” birth in rhinoceros is needed, but as of this time has not been published. Thus some of the data analysis in this paper is limited to generalizations for rhinoceros approved by the AZA Rhino TAG. Therefore the following is considered a “normal” delivery.

Though rhinos may be in labor for extended periods of time, once the feet are showing the calf comes quickly, usually in less than one hour. If a calf’s feet are presented and the dam takes more than one hour to deliver, it often results in a compromised, stillborn or dead calf. A normal delivery is one that results in the birth of a calf within 60 minutes of feet presentation, with no additional factors. A dystocia birth is any birth that does not follow the normal parameters.

For many species amniotic sac rupture prior to delivery is not an issue. Horses often deliver after the amniotic sac has ruptured delivering a healthy live foal. The difference may be that an anterior delivery is considered normal as opposed to posterior. If the front feet and head are already out of the birth canal, the foal has the ability to breathe on its own during delivery. Since rhinoceros are able to deliver normally both anterior and posterior, premature amniotic sac rupture may contribute to the delivery of a stillborn calf. For the purposes of this study amniotic sac rupture is considered as a possible reason for stillbirth and thus significant.

More data on not only greater one-horned rhino birthing but all rhino birthing will better answer the questions of the relationships between birth presentation, premature amniotic sac rupture and stillbirths in rhinoceros. Through the International Rhino Keeper Association a request for information was sent to members to send videos of their rhino births adding to the data pool for study. While several institutions have recorded births, few are willing to share footage of the birth in a public format, losing control over who observes such recordings. A compromise is to ask each institution to use set criteria for analysis and provide data as opposed to provide videos. This information is still being gathered and will be added to the data in the future to hopefully gain a better understanding of stillbirths in rhinos.
References


Foaling Guide, Maria S Ferrer, DVM, MS, DACT, Veterinary Medical Teaching Hospital, Kansas State University.

“Relationships Among Birth Presentation, Amniotic Sac Rupture and Stillbirths in Rhinoceros.”

Jane Kennedy, Lead Keeper, San Diego Zoo Safari Park
North American Regional Studbook Keeper, Greater One-horned Rhinoceros
Vice-president International Rhino Keeper Association
Rhino Births

- 41 years-173 Births
- No Northern White Rhino
- 93 Southern White Rhinos, 3 Generations
- 15 Eastern Black Rhinos, 5 Generations
- 65 Greater One-horned Rhinos, 7 Generations
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<tr>
<td>1972-1.1</td>
<td>From SDZ</td>
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<tr>
<td>1975-1st</td>
<td>Calf born</td>
</tr>
<tr>
<td>2013-65th</td>
<td>Calf, a stillbirth</td>
</tr>
</tbody>
</table>

- Jakichu and Jontu, our 55th calf
- 16 Stillbirths
- 12 of 27 females in NA Studbook have had a stillbirth
Stillborn calf March 2012
SDZSP GOHR Data

- 5 of 6 Anterior live births
- 1980s UNK live birth
- 13 March 1994-Jumia live birth
- 4-Jan-96 –Jumia live birth
- Dec 2009-Raji live birth
- 15-May-2011-Jatri-dystocia, head first no legs, both die
- 20-Jan-12 –Alta live birth

- 5 of 8 Posterior live births
- 24-Mar-75-Jaypuri live birth that died
- 28-May-87-Jaypuri live birth
- 27-Jan-90-live birth
- 23-Nov-97-Godavari live birth
- 25-Jan-05-Gari live birth
- 20-October-2010-Asha stillbirth
- 15-Mar-12 -Asha stillbirth
- Jan 5 2013-Kaya stillbirth

20-Nov-97 Gainda stillbirth likely posterior, but unconfirmed

Bolded are videoed
Youtube Data

- 3 GOHR, 1 anterior, 2 posterior
- 4 SWR, 2 anterior, 2 posterior
- 1 Black, 1 anterior
- 1 Sumatran, 1 posterior

- All Live births, 4 anterior, 5 posterior
Asha October 20, 2010

Delivered stillborn male calf after 3 hours, 169 lbs
Kaya January 2013

Stillborn, posterior, sac ruptured
Kaya’s stillborn January 6, 2013

Delivered stillborn male calf after at least 50 minutes
Gainda 1997 stillborn
Gainda-excessive fluid release
Gainda 1997
UNK, stillborn, sac ruptured?
Gainda
posterior? sac rupture?
Unknown Dam 1980’s anterior, live birth, sac intact
Jumia 13 March 1994
anterior, live birth, sac intact
Jumia 4 Jan 1996
anterior, live birth, sac intact
Alta Jan 20, 2012
anterior, live birth, sac intact
Netherlands GOHR-2008
anterior, live birth, sac intact
Netherlands GOHR 2011
posterior, live birth, sac intact
Netherlands GOHR 2012
posterior, live birth, sac intact
Sumatran Rhino
Cincinnati
SDZSP SWR Ujima posterior, live birth, sac intact
SDZSP SWR Kacy
anterior, live birth, sac intact
German SWR
anterior, live birth, sac intact
Taipei SWR
posterior, live birth, sac intact
Israeli Rhino
anterior, live birth, sac intact
German Black Rhino anterior, live birth, sac intact
SZDSP Black Rhino Jeri posterior, sac ruptured, live birth
Chester Zoo Kitani’s Births

Asani
Male
Born: 29/10/08

Breech birth
Male
Born: 06/07/12
Chester Zoo Chanua’s Birth (calving pen)
Andatu
Combined Rhino Birthing Video Data

SWR-7
4 anterior, 2 posterior, 1-UNK
All live births, sac intact

Black rhinos-5
2 anterior, 3 posterior
4 live births
1 Posterior had sac rupture
1 posterior dystocia stillbirth

Sumatran-3
1 anterior, 2 posterior
All live births

- GOHR births-14
  - 5 anterior, 8 posterior, 1-unconfirmed posterior
  - 5 anterior, 4 posterior-live births, sac intact
  - 4 posterior-stillbirths
  - 1 suspected posterior stillbirth

TOTAL-29 births
13 anterior, 13 live birth
15 posterior, 10 live birth, 5 stillbirth
1 suspected posterior stillbirth
# Combined Rhino Birthing Data

<table>
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<tr>
<th>Species</th>
<th>Anterior</th>
<th>Posterior</th>
<th>Live Births</th>
<th>Stillbirths</th>
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<tr>
<td>SWR-7</td>
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<td>2</td>
<td>4</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>All live births</td>
<td></td>
</tr>
<tr>
<td>Black rhinos</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1-Posterior had sac rupture ~15 minutes</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>4-live births</td>
<td>1 posterior dystocia stillbirth</td>
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<tr>
<td>GOHR births</td>
<td>8</td>
<td>7</td>
<td>12</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td>8 live birth</td>
<td>1 suspected posterior stillbirth</td>
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<tr>
<td>Sumatran-3</td>
<td>1</td>
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<td>3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>All live births</td>
<td></td>
</tr>
</tbody>
</table>
Combined Rhino Birthing Data

TOTAL-36 births

15 anterior
  14 live births, all sac intact
  1 stillbirth, dystocia, fetotomy-SDZSP

19 posterior
  12 live births
  11 sac intact, 1 amniotic sac ruptured
  6 stillbirths, all amniotic sac ruptured
  1 born dead, dystocia, fetotomy-Chester

1 suspected posterior stillbirth
1 unk live birth (SWR video-Dublin)
Relationships Among Birth Presentation, Amniotic Sac Rupture and Stillbirths in Rhinoceros

Birth presentation may be significant
All 5 videoed stillbirths posterior presentation

Amniotic sac rupture may be significant
All 7 stillbirths had sac rupture
(All but 1 live birth had sac intact)

More research is needed
What I need help with

• Collect more data on other rhino births
• Expand the collaborative team
• Further develop an accurate assessment plan of data
• When the members of an organization work together, important questions are answered
• We can answer this question!
Special Thanks to all of you and...

- My Husband, Big Eddie
- East Crew, Best Crew Ever!
- San Diego Zoo Global Employees
- Collections, Husbandry and Sciences Department SDZSP
- Institute for Conservation Research
- Dr. Lance Miller
- Randy Rieches-Henshaw Curator, SDZSP
- Lance Aubery, SDZSP
- Andy Blue, SDZSP
- Chester Zoo-Mark Cleave, Tim Hamilton
- Cincinnati Zoo
- Dr. Terri Roth, Dr. Monica Stoop,
- Leah Winstead-Intern video observer
- Buffalo Zoo-Joe Hauser

Ask the animals, they will teach you...JOB 12:7
Why we do this
Thermoregulation in the African Elephant (*Loxodonta africana*) and possible effects on fertility.

R.L. Ball¹, M.W. James¹, M.J. Schotsman², Brandon Laforest², J.L. Atkinson², E.J. Finegan² and S.P. Miller²

Tampa’s Lowry Park Zoo¹ and Department of Animal and Poultry Science University of Guelph
Elephants

- Largest living land animal
  - 5,000 - 6,000 kg
- Habitat
  - African savanna
  - Heat gained from solar radiation, landscape, metabolic processes
  - Captive habitats
- relatively low metabolic rate (/kg body weight) and low surface area:body mass
- heat which is absorbed becomes harder to lose because of the elephant’s low surface area
  - Need a way to shed excess heat
Temperature’s Effect on Fertility

• Heat Stress
  – Caused by rise in core body temperature
  – Contributing climatic factors (Gwazdauskas, 1985):
    • Temp
    • Humidity
    • Radiation
    • Wind
  – Studies in dairy cattle have shown (Jordan, 2003):
    • Increase in body temperature of ~0.9°F can cause a decline in conception rate of 12.8%
    • Negative effects can be identified from 42 d prior to and 40 d after insemination
    • Heat stress does not have to last for months to have profound negative impacts, but can occur in days, even in temperate climates
Heat Stress in Males

- oxidative stress and decrease in fertility

MINI REVIEW

Do heat stress and deficits in DNA repair pathways have a negative impact on male fertility?

Catriona Paul\(^1\), David W. Melton\(^2\) and Philippa T.K. Saunders\(^{1,3}\)

\(^1\)MRC Human Reproductive Sciences Unit, Queen’s Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK;
\(^2\)Sir Alastair Currie Cancer Research UK Laboratories, Molecular Medicine Centre, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK
Heat Stress in Females (citations?)

- alter the duration of estrus
- colostrum quality
- conception rate
- uterine function
- endocrine status
- follicular growth and development
- luteolytic mechanisms
- early embryonic development
- fetal growth

- the only known method for increasing fertility in heat-stressed cows is to **cool the cow**
- hormonal treatments have been tested for increasing fertility of heat-stressed cows but none of them have been shown to consistently cause an increase in fertility of heat-stressed cows
Possible Strategies to Avoid Heat Stress

• Behavioral
  – Seek shade under trees (Kinahan et al., 2007a)
  – Mudding/wallowing
  – Ear-flapping (Phillips and Heath, 1992)
    • two to three degrees Celsius (°C) between the temperature of the blood entering the ear in the arteries and the blood leaving in the veins.
  - Evaporation through the skin (Wright and Luck, 1984)
Possible Strategies to Avoid Heat Stress

– Adaptive Heterothermy
  • Some mammals in hot, arid areas are capable of allowing their core body temperature to rise
    – instead of jeopardizing their water balance and energy reserves to cool off
    – They then lose this excess heat during the night, using the cool sky and landscape as heat sinks
  • Elephant core body temperatures may vary as much as 5°C (9°F) (Elder and Rodgers, 1975)
  • Use a countercurrent blood vessel arrangement known as a rete to cool blood flow to the brain
Thermoregulation by the African Elephant, *Loxodonta africana*

M.J. Schotsman, J.L. Atkinson, E.J. Finegan and S.P. Miller
*Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario*
*EMA 2008*

**Abstract**

As large mammals in hot, arid environments, African elephants face difficulties losing excess heat gained during the day. They may store heat gained during the day, and release it at night through vasodilation in their ears and sides. Three African elephants were observed over six nights and their core body, side, and ear temperatures were measured. Side and ear temperatures decreased faster on cold nights (slopes of -0.11 to -0.21) than warm nights (slopes of -0.03 to -0.21). Both side and ear temperatures were more variable on warm nights. Core body temperatures varied by as much as 4 degrees Celsius. These findings indicate heat storage during daylight hours and heat loss overnight.

EMA 2008 Orlando
Hypothesis

• Elephants store heat during the day instead of using energy and water to keep body temperature stable
• Skin temperatures will stay high early in the night
• Ear temperatures will be high for some part of the night
• Core body temperatures will go down over the course of the night
Methods

• Study animals
  – 3 female African elephants at the Toronto Zoo
  – 2 acre paddock
  – Three observation sites used
Methods

• **Procedure:**
  – Every 15 minutes weather data taken
    • Solar radiation, background longwave radiation, cloud cover, relative humidity, windspeed, and air temperature
  – Infrared images taken every 15 minutes
    • Side-on, ears visible
  – **Images also taken whenever urinations occurred as a measure of core body temperature**
    • Benedict and Lee (1936)
  – Observations made on 10 nights through June and July from sunset to sunrise
Thermal Imaging

• Infrared/longwave radiation
  – Emitted by anything with a temperature over absolute zero (-273 °C)

• Thermal imaging camera (FLIR Thermacam)
  – Uses infrared radiation emitted by a surface to determine the temperature of the surface
Methods

•Outlined sides and ears, took average temperature of both areas
Results

• Huge number of pictures
  – More than 2400 images to analyze!

• Complications...
  – Animals not always in view, may be lying down or leaning against walls
Focus on three elephants

• Why these three?
  – Most visible
  – Little time spent lying down or behind obstructions
  – Frequent visible urinations

• Data from 6 nights, 3 “warm” and 3 “cold”
Discussion

- Decrease in side, ear, and urine temperatures over the course of the night
  - Ear temperatures were more variable on warm nights than cold nights \((R^2 = 0.15 - 0.87, \text{Cold nights } R^2 = 0.40 - 0.91)\)
  - Side temperatures were more variable on warm nights than cold nights \((R^2 = 0.14 - 0.84, \text{Cold nights } R^2 = 0.61 - 0.94)\)
  - Urine temperatures indicate that core body temperature can decrease as much as \(4.9^\circ C (8.8^\circ F)\) over the course of the night
  - Surface temperature tends to decrease more rapidly on cold nights
Conclusions

• Managed elephants do have a highly variable core body temperature.
• This fluctuation may be more than wild elephants (Kinahan 2007)
• Initial support for adaptive heterothermy in African elephants
• **Elephants may require opportunities to thermoregulate**
Asian Elephants-BGT
Brandon Laforest, 2009 Guelph
Faculty of Environmental Studies, York University, Toronto

• actively retain heat the longest in their trunk
• The body to a lesser degree to dissipate heat
• The ears, smaller compared to body size than African ears, cool most quickly
Is heat stress present in managed elephants and how?

Indoor housing

Obesity
Reproductive Capabilities of the Managed Population

- Asian Elephants ♀’s (ages 10-40)
  - 21/56 (37.5%) are considered non or post-reproductive
- African Elephants ♀’s (ages 10-40)
  - 71/102 (69%) are considered non or post-reproductive

- Why such a high rate of infertility?
  - Could be several contributing/intertwining environmental factors
    - Heat Stress
    - Obesity
    - Diet

(AZA SSP Population Analysis, 2011)
How can we reduce heat stress in managed elephants?

- Exhibit Design
  - Shade
  - Access to water features/wallows
  - Reflective features
How can we reduce heat stress in managed elephants?

• Create circumstances that allow for adaptive heterothermy
  – Access to outdoor space overnight
• Diet
• the only known method for increasing fertility in heat-stressed cows is to
  cool the cow
Further Investigations

• Verify that elephants exhibit adaptive heterothermy
  – Urine temps as core validation in progress

<table>
<thead>
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<th>BARN TEMPERATURE, °C.</th>
<th>WEIGHT OF URINE COLLECTED, KG.</th>
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<td>11:02 P.M.</td>
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<td>3.40</td>
<td>35.9</td>
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Further Investigations

• Association between infertility and obesity
  – Endocrine or heat or both??

• Temperature management and semen collection
  – Indoor bulls??

• Study heat loss indoor overnight
White rhinoceroses

- Adaptive thermoregulatory behaviors, which limit heat gain during the hottest hours
- Large diurnal temperature variation
  - (Allbrook et al., 1958)
- Local blood flow to the body surface
  - Thermal windows
  - Epidermis 1mm, well-developed subjacent vascular bed
White rhinoceroses

• Do white rhinos exhibit adaptive heterothermy?
  – life history as an arid-zone megavertebrate
• If not, are they still given opportunity to thermoregulate (dissipate heat)?
• Do they acquire more heat in captive situations?
• If no, then is heat stress contributing to infertility?
White rhinoceros

- Preliminary observations suggest no adaptive heterothermy
- Shade a predominant behavioral cooling mechanism
White rhinoceros reproduction success

- San Diego Safari Park
- Busch Gardens Tampa
- Western Plains Dubbo
- Lowry Park Zoo
- Space
- Housing
  - Indoors vs outdoors
- Expand thermal research with white rhinos
  - Associations with repro success
Acknowledgments

- University of Guelph
- Busch Gardens Tampa Elephant Team
- TLPZ Elephant and Rhino Staff

Katie L. Edwards, Susanne Shultz, Mark Pilgrim and Susan L. Walker
Ex situ conservation
EEP population of black rhino
EEP population of black rhino
### What could be limiting growth?

<table>
<thead>
<tr>
<th></th>
<th><strong>Ex situ</strong></th>
<th><strong>In situ</strong></th>
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<tbody>
<tr>
<td><strong>Last 10 year period</strong></td>
<td></td>
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</tr>
<tr>
<td>Females breeding per annum</td>
<td>11.3%</td>
<td>23.7%</td>
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<tr>
<td>Females aged 5-32 produced offspring</td>
<td>40.7%</td>
<td>52.9%</td>
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<tr>
<td>Calves per breeding female</td>
<td>1.54</td>
<td>2.11</td>
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<table>
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<tr>
<th></th>
<th><strong>Previous 10 year period</strong></th>
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<tr>
<td>Females breeding per annum</td>
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<td>Calves per breeding female</td>
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</tbody>
</table>
What could be limiting growth?

Non-proven

42.1% 48.6%
Differences in reproductive success? 
Hormones and reproductive success

Glucocorticoids
- Proven males had higher testosterone than non-proven males
- No relationship between testosterone and glucocorticoids
4 types of cyclicity pattern observed
No evidence of seasonality
Long cycle types more commonly observed in non-proven females
Glucocorticoids were higher during long cycle types

- Especially in non-proven females
What other factors could be involved?
Non-proven females scored higher BCS than proven females
Non-proven females were less likely to express regular behavioural signs of oestrus.
<table>
<thead>
<tr>
<th></th>
<th>‘Almost always behaves the same’</th>
<th>‘Sometimes can be unpredictable’</th>
<th>‘Very unpredictable’</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td><img src="image1" alt="Male Rhino" /></td>
<td><img src="image2" alt="Male Rhino" /></td>
<td><img src="image3" alt="Male Rhino" /></td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td><img src="image4" alt="Female Rhino" /></td>
<td><img src="image5" alt="Female Rhino" /></td>
<td><img src="image6" alt="Female Rhino" /></td>
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</tbody>
</table>

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<thead>
<tr>
<th><strong>GC</strong></th>
<th><strong>PROVEN</strong></th>
<th><strong>NON-PROVEN</strong></th>
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<tbody>
<tr>
<td></td>
<td><img src="image7" alt="Green Arrow" /></td>
<td><img src="image8" alt="" /></td>
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</tbody>
</table>
Extrinsic factors
Summary

• Sub-optimal reproduction limiting population growth
• Hormone differences between breeding and non-breeding rhinos
• In males, testosterone differences unrelated to extrinsic factors
• In females, erratic cyclicity is relatively common
• Long cycle types may be indicative of a problem – glucocorticoids
• Body condition related to reproductive success
• Expression of oestrus can be unreliable, especially in non-proven females
• Temperament related to glucocorticoids and reproductive success
Any Questions?
Improving welfare of captive Asian elephants in Kerala, India

T.P. Sethumadhavan, K.C. Panicker, R. Radhika

1. T.P. Sethumadhavan, Head, Publications, Directorate of Entrepreneurship, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur-680651, Kerala, India. 2. K.C. Panicker, Professor and Head (Retd), Department of Veterinary Parasitology, Kerala Agricultural University & Secretary, Elephant Welfare Association, Thrissur, India 3. R. Radhika, Assistant Professor Department of Veterinary Parasitology, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur-680651, Kerala, India.

Abstract

Improving the welfare of captive Asian elephants in India is emerging as an important conservation issue. Improving elephant welfare is one of the research priorities of scientists, policy makers and researchers. This study was done in Kerala state, the southern state of India where elephants are used widely for festivals. Tuskers are used for processions and festivals in Temples and churches. Recent findings revealed that due to poor elephant management, 400 human lives have been lost during the last three and a half decades in India, of which more than 90 per cent of the victims were mahouts. It is clear that poor elephant management practices cause elephant aggression toward mahouts.

This study was conducted in the southern state of India, Kerala, which occupies only 1.13 percent of the geographic area of the country. The state has 600 captive Asian elephants. As part of the study 100 captive Asian elephants were selected to determine the cause of elephant aggression and to formulate measures to improve captive elephant welfare. Variables like breeding, feeding, management, season, age, musth, behavior, transportation and diseases were identified. Major interventions affecting the above variables were also identified and a SWOT analysis was done. Major indicators of poor elephant welfare included reduced chances for breeding, unscientific feeding and management methods, prolonged standing during festivals, unscientific musth management, poor musth forecasting system, heat stress, poor transportation and disease management.

Based on the above findings, a system was formulated to improve elephant management after taking into account the major variables identified including best feeding and management practices, musth forecasting system, scientific disease control system and best management practices during transport and festivals.

The formulated scientific management system was administered on 100 captive Asian elephants during the festival season of 2012-2013. During the festival season, when the elephants are compelled to stand more than 6 hours, measures were taken to frequently give watery vegetables like cucumber and watermelon. In order to reduce heat stress the elephants were allowed to walk in shady places. Wet gunny bags were placed underneath their feet. Shamianas were made over their standing space to protect them from scorching sunlight. A twelve hour rest period was made compulsory for elephants before moving to the next festival. Mahouts were given training in scientific management practices. As part of transportation norms, elephants were allowed to walk only 20 kms per day during the morning and evening hours. For distances to be traveled beyond 20
Km, travel by trucks was made mandatory as per the State’s captive elephant management rule.

The study findings revealed that best captive elephant management practices could reduce 90 percent of the elephant aggression toward humans. In order to reduce stress, a scientific feeding & management program and disease control during musth also needs proper attention. There is a positive correlation between season and incidence of musth. A musth forecasting system will help to reduce elephant aggression toward humans and maintain appropriate elephant welfare.

**Introduction**

Among range countries, measures to improve welfare of captive Asian elephants is an important conservation issue. Improving the welfare of captive Asian elephants in India is emerging as an important conservation issue. Improving elephant welfare is one of the research priorities of scientists, policy makers and researchers. This study was done in Kerala state, the southern state of the country where elephants are used widely for festivals. Tuskers are used for processions and festivals in Temples and churches. Recent findings revealed that due to poor elephant management, 400 human lives have been lost during the last three and a half decades in India, of which more than 90 percent of the victims were mahouts. It is clear that poor elephant management practices cause elephant aggression toward mahouts.

**Materials and methods**

This study was conducted in the southernmost state of India; Thrissur in Kerala which occupies only 1.13 percent geographic area of India. Human-captive elephant interaction is primarily observed in regions of the state where tuskers are used for festivals and processions. There are certain festivals where up to 70 animals at a time will be used for a procession. Kerala has more than 600 captive Asian elephants. As part of the study, 100 captive Asian elephants were randomly selected to analyse the animal welfare issues and to find suitable measures to improve the situation.

Different variables like feeding, management, season, age, musth incidence, behavior, transportation, breeding and diseases were identified. Data was collected from different stakeholders such as veterinarians, mahouts, elephant owners, festival organizers, policy makers and elephant lovers with the help of standardized interview forms. Focus group discussions were also conducted. Major interventions affecting the above variables were identified and a SWOT analysis was conducted. The societal drivers of attitudes to elephant issues can be analysed in terms of a ‘V-STEEP’ (Values, Social, Technological, Economic, Environmental and Politico-legal) framework of Rogers (2005), an extension of the SEEP framework of Campbell & Olson, 1991. Appropriate management protocols were developed to improve the welfare of captive Asian elephants.
Results and discussion

Major elephant welfare issues identified were stress due to overwork during festival season, poor chances of breeding, unscientific feeding and management, prolonged standing in festivals, improper musth management, poor musth forecasting system, heat stress & climatic variations, poor transportation and disease management.

SWOT analysis

<table>
<thead>
<tr>
<th>Strength</th>
<th>Weakness</th>
<th>Opportunities</th>
<th>Threat</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. 600 Captive Asian Elephants</td>
<td>2. Major festivals are during December to May</td>
<td>2. Musth forecasting system</td>
<td>2. Increasing musth incidents during winter</td>
</tr>
<tr>
<td></td>
<td>6. Climatic variation</td>
<td></td>
<td>7. Traditional festival practices</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>8. Unpredictable behavior of elephants</td>
</tr>
</tbody>
</table>

Based on the above findings and different variables identified, management protocols were formulated to improve the welfare of captive Asian elephants. Major variables include best feeding and management practices, musth forecasting system, scientific disease control system and best management practices during transport and festivals.

The formulated scientific management practices were administered on 100 captive Asian elephants during the festival season of 2012-2013. During the festival season, when the elephants were standing more than 6 hours, measures were taken to frequently give watery vegetables like cucumber and watermelon. They were allowed to walk in shady places during the festivals. Wet gunny bags were placed underneath their feet. Shamianas were made on their standing space to protect them from scorching sunlight. A twelve hour rest period was made compulsory for elephants before proceeding to the next festival. Mahouts, veterinarians and media persons were given training in scientific management practices. Elephants are only allowed to walk 20 kms per day and only during morning and evening hours. For distances beyond 20 km, the
elephant must be transported by trucks and this regulation was made compulsory as per the Captive Elephant Management Rule. The presence of veterinarians and forest officials was made compulsory during festivals to monitor the situation.

**Management protocols**

1. Elephants require adlibitum fodder/ green leaves and water just prior to festival procession/parade.
2. Elephants require a rest period of 12 hours before the next festival procession.
3. During festivals, elephants were compelled to stand under scorching sunlight and on overheated floors causing stress and foot lesions. Sufficient shade should be provided at the procession venue. Frequent sprinkling of water on the floor and foot was suggested.
4. During transportation, elephants cannot be allowed to walk more than 20 Km at a time. It should be done during morning and evening hours. Trucks should be used for transportation for a distance of more than 20 Km. Sufficient care should be taken while transporting elephants in trucks.
5. During festivals where elephants are compelled to stand more than 6 hours at a time, succulent fruits like watermelon, banana and pineapple should be given.
6. A registered veterinary practitioner should examine elephants before they are put to use in festivals.
7. Animals showing pre-musth, musth and post musth symptoms should not be allowed to participate in the festivals.
8. Based on age, season and previous musth incidents, a musth forecasting system was developed. This can be used as a screening process.
10. Awareness programmes for public, festival organizers, students and elephant lovers are suggested.
11. Registered veterinary practitioners and forest officials should monitor the welfare of animals during festivals where elephants are put to use for procession.

The study findings revealed that best captive elephant management practices could reduce by 90 percent the elephant aggression toward humans. In order to reduce stress, scientific feeding & management, disease control and management during musth needs proper attention. There is a positive correlation between season and incidence of musth. Musth forecasting system based on previous incidences of musth, age and season will help to reduce elephant aggression toward humans and maintain a sustainable elephant welfare system. Social attitudes and constructs among stakeholders should be influenced in ways that allow greater sharing of information and values on elephant management.

**Literature cited**-

1. Chandrasekharan. K (2002); specific diseases of Asian elephants; *J. Ind Vet Assoc*; Special issue on elephants, 7(2); pp 31-34.
3. Harry C Biggs, Rob Slotow 2005,Towards integrated decision making for elephant management
   Pretoria.

Authors are thankful to the Elephant welfare association, Thrissur, Kerala and Kerala Veterinary and Animal Sciences University, Kerala, India for providing facilities for conducting the study.
Improving welfare of captive Asian elephants in Kerala, India

T.P.Sethumadhavan, K.C.Panicker & R.Radhika
Kerala Veterinary & Animal Sciences University, India
www.kvasu.ac.in
Asian elephants occur in isolated populations in 13 range States, with an approximate total range area of almost 880,000 square kilometers equivalent to only one-tenth of the historical range as defined by the IUCN. Today Asian elephants occur in Bangladesh, Bhutan, India, Nepal, Sri Lanka, Cambodia, China, Indonesia, Lao People’s Democratic Republic, Malaysia, Myanmar, Thailand, and Viet Nam. Feral populations occur on some of the Andaman Islands in India. Recent reports from across the 13 Asian elephant range States suggest that there are between 39,500 and 43,500 wild Asian elephants. In addition, there are approximately 13,000 domesticated (working or former working) elephants in Asia.
Asian elephants occur in isolated populations in 13 range States, with an approximate total range area of almost 880,000 square kilometers equivalent to only one-tenth of the historical range as defined by the IUCN.
Geographic distribution

- Asian elephants occur in Bangladesh, Bhutan, India, Nepal, Sri Lanka, Cambodia, China, Indonesia, Lao People’s Democratic Republic, Malaysia, Myanmar, Thailand, and Viet Nam. Feral populations occur on some of the Andaman Islands in India. Recent reports from across the 13 Asian elephant range States suggest that there are between 39,500 and 43,500 wild Asian elephants. In addition, there are approximately 13,000 domesticated (working or former working) elephants in Asia.
Indian Elephant Population Figures

- Elephant Range: 110,000 km²
- Country Ranking: 2nd of 13
- Wild elephants: 23,900
- Total Captive Population: 3,500
  Country Ranking: 3rd
Domesticated Asian Elephants in India - Current status

3300 domesticated Asian Elephants
75% owned by individuals
6 Percent by temples
2 percent by zoos
3 percent by circuses
14 percent by state forest departments
Purpose of rearing

39% are used for logging
10% for transportation
16% for Tourism
3% for entertainment (Circus & Zoos)
20% for ceremonial purposes
2% for agriculture
7% for begging
Kerala Scenario

A tiny state with 1.13% geographic area of the country
600 captive Asian elephant population
Mostly Tuskers are used for festivals, processions and work
Human-Elephant contact during festivals
Mahouts and the elephant
Tuskers are used during festivals
Temple festival in Kerala-Thrissur Pooram
Festival procession through the crowd
Elephant lovers as Friends of Elephants
Young Mahout
Traditional Custom of worship-anayooottu
Feeding of Elephants
Elephant aggression towards Mahouts
Elephant beyond control
Recent findings revealed that 400 human lives were lost due to elephant aggression during the last three and a half decades in Kerala, India. Of which more than 90 Percent victims are mahouts.
Objectives of the study

1. To find out the cause and to formulate the required measures to reduce Elephant aggression.
2. To formulate management protocols and musth forecasting system to reduce increasing incidence of aggressive behavior in elephants.
Materials and methods

As part of the study 100 domesticated/ captive Asian elephants were randomly selected to find out the cause and to formulate measures to reduce the aggressive behavior among elephants.
Different variables studied

- Different variables like feeding, management, season, age, musth incidence, behavior, transportation, breeding and diseases were identified.
- Data were collected from stakeholders involved in elephant welfare like veterinarians, mahouts, elephant owners, festival organizers, policy makers and elephant lovers with the help of interview schedule.
- Focus group discussions were also conducted. Major interventions affecting above variables were identified.
- SWOT analysis was done.
- Based on the findings management protocols were developed to reduce the increasing incidence of aggressive behavior among elephants.
Results and discussion

Major elephant welfare issues are stress due to over work during festival season, poor chances of breeding, unscientific feeding and management, prolonged standing in festivals, improper musth management, poor musth forecasting system, heat stress & climatic variations, poor transportation and disease management.
Strength & Weakness

- Literacy
- 600 Captive Asian Elephants
- Captive Elephant management rule 2003
- Network of veterinary institutions
- Media awareness
- Friends of Elephant culture

- Over work during festive season
- major festivals are during December to May
- Poor chances of breeding
- Improper feeding and watering
- Poor musth management
- Transportation stress
- Climatic variation
Opportunities & Threat

- Captive elephant management rule
- Musth forecasting system
- Awareness programmes at the institutional level
- Good mahout practices
- Management protocols
- Positive attitude towards elephant welfare

- Frequent climatic variation
- Increasing musth incidents during winter
- Poor management practices
- Unscientific mahout practices
- Improper implementation of captive elephant management rule
- Cruelty on elephants
- Over work during a particular season
- Traditional festival practices
- Unpredictable behavior of elephants
Management protocols

Best feeding and management practices, musth forecasting system, scientific disease control system and best management practices during transport and festivals were developed.
Existing management protocols were modified and applied new scientific management protocols on 100 captive Asian elephants during the festival season January to May 2013.
Based on the SWOT analysis remedial measures were identified in breeding, feeding, management, season, age, musth incidence, musth forecasting, Mahout training, behavior, over work, cruelty, transportation and disease control in tune with captive elephant management rule 2003.
Management protocols

• Elephants require adlibitum fodder/ green leaves and water just prior to festival procession/parade.
• Elephants require a rest period of 12 hours before the next festival procession.
• During festivals elephants were compelled to stand under scorching sunlight and on overheated floor. This causes stress and foot lesions. Sufficient shade should be provided at the procession venue. Frequent sprinkling of water on the floor and foot were suggested.
• During transportation, elephants cannot be allowed to walk more than 20 Km at a time. It should be done during morning and evening hours. Trucks should be used for transportation for a distance of more than 20 Km. Sufficient care should be taken while transporting elephants in trucks.
• During festivals where elephants are compelled to stand more than 6 hours at a time, succulent fruits like watermelon, banana and pineapple should be given.
• A registered veterinary practitioner should examine elephants before they are put to use in festivals.
• Animals showing pre-musth, musth and post musth symptoms should not be allowed to participate in the festivals.
• Based on age, season and previous musth incidents, a musth forecasting system is developed. This can be used as a ready reckoner for screening process.
• Mahouts must be trained as per captive elephant management rule 2003.
• Awareness programmes for public, festival organizers, students and elephant lovers are suggested.
• Registered Veterinary practitioners and forest officials should monitor the welfare of animals during festivals where elephants are put to use for procession.
Musth forecasting

Based on

Season
Behavior
History

Swelling of the temporal gland
Oedema around the perineal region

Behavior towards mahouts
Symptoms
Hormonal assay
5. During festivals where elephants were compelled to stand more than 8 hours at a time, succulent fruits like watermelon, banana and pineapple should be given.

6. A registered veterinary practitioner should examine elephants before they are put to use in festivals.

7. Animals showing pre-musth, musth and post musth symptoms should not be allowed to participate in the festivals.

8. Based on age, season and previous musth incidents, a musth forecasting system was developed. This can be used as a ready reckoner for screening process.

9. Mahouts must be trained as per captive elephant management rule 2003.

10. Awareness programmes for public, festival organizers, students and elephant lovers were suggested.
The management protocols were applied to elephants during the peak festival season from January to May 2013. As a result, the incidence aggressive behavior of elephants could be reduced up to 90 percent in the state.

Study revealed that there exists a positive correlation between Elephant's aggressive behavior and poor feeding and management practices.
Conservation issue

- A future for Asian elephants ensures a future for other species and wild spaces
Initiatives for elephant welfare

• Animal welfare clubs at school level
• Capacity building for Veterinarians, and Forest officials
• Skill development programmes for Mahouts
• Awareness programmes for School children and public
• Knowledge dissemination on elephant welfare through print, electronic and web media
• Research elephant welfare issues
Authors are thankful to the Kerala Veterinary and Animal Sciences University, Thrissur, Kerala, India for providing facilities for conducting the study.
Thank You
Relationship between management, adrenal activity and reproduction in a captive group of female Asian elephants (Elephas maximus)

Jess Trotter, Katie Edwards, Martin Jones, Hanspeter Steinmetz, Susan Walker
In Captivity Welfare and Sustainability is Key
Introducing the Herd

**Matriarch**
- Sheba

**Adults**
- Jangoli
- Thi
- Birma

**Sub Adult**
- Sithami

**Juvenile**
- Sundara
Chester’s Breeding Programme

Period 1
Period 2
Period 3
Period 4

Jangoli
Sithami
Thi
Sundara
Birma

Progesterone metabolite
(ng/g faeces)
Data Collection

- Study Period: March 2008 to June 2009
- Faecal samples analysed for progesterone and corticosterone metabolites
- Management factors: Animal Records Keeping System (ARKS) and the elephant keepers’ daily diaries
Data Collection

• Study Period: March 2008 to June 2009

• Faecal samples analysed for progesterone and corticosterone metabolites

• Management factors: Animal Records Keeping System (ARKS) and the elephant keepers’ daily diaries
Questions

1) Were management factors related to adrenal activity?

2) Was adrenal activity related to acyclicity?
Training

- In 4 individuals no effect of routine training on adrenal activity

- In 1 individual intensive training was related to higher adrenal activity
In 4 individuals no effect of routine footcare on adrenal activity

In 1 individual intensive foot care was related to higher adrenal activity
Presence of Matriarch

MATRIARCH: Sheba

ADULTS: Jangoli, Thi, Birma

SUB ADULT: Sithami

JUVENILE: Sundara
Adrenal Activity and Acyclicity

During acyclicity:

- For 1 female adrenal activity was lower during the acyclic period than during cycling
- For 2 females there was no difference

Prior to acyclicity:

- For all 3 females, adrenal activity was not elevated during the cycle prior to acyclicity
Findings

- What about acyclicity?

- Pregnant females – herd was highly synchronised prior to this study, and we cannot rule out the influence of a behaviourally dominant female conceiving.......
Findings

• Management recommendations made after this study:
  • To minimise separation of the family group for training
  • To not separate the matriarch from the group
  • Refurbishing enclosures with sand to benefit foot health
  • Installing natural features to provide a more stimulating environment
Any Questions?
SOCIAL AND REPRODUCTIVE BEHAVIOUR OF CRITICALLY ENDANGERED NORTHERN WHITE RHINOCEROS (CERATOTHERIUM COTTONI) IN A ZOOLOGICAL GARDEN

Ivana Cinková,* Vítězslav Bičík

Department of Zoology and Lab of Ornithology, Faculty of Science, Palacký University, 17. listopadu 50, 771 46 Olomouc, Czech Republic; e-mail: ivanacinkova@centrum.cz

Group composition may be an important factor for optimal welfare and reproduction of socially living mammals. Northern and southern white rhinos are the most social of all rhinoceros species, and females, sub-adults and juveniles live in groups. However, white rhinos in zoological gardens are often kept in small numbers and they cannot change their companions as often as they do in the wild. White rhinos have low reproductive success in captivity and social interactions between the animals, especially their increased agonistic behaviour, might be one of the reasons. Therefore, appropriate group composition and/or a change of social relationships in white rhino herds might have a positive effect and increase reproduction of the captive rhinos. However, studies experimentally investigating the influence of changes in group structure on the social and reproductive behaviour of captive rhinos are missing. The northern white rhino is currently on the brink of extinction with only seven animals known to survive. We studied the social and reproductive behaviour of a group of northern white rhinos (one male, five females) in zoological garden Dvůr Králové in 2005. The most often observed agonistic activities among the animals were threat, snarl and clash of horns. The agonistic behaviour was most frequently directed from the females towards the male and agonistic activities between the females were rarely recorded. In the middle of our study, one of the females (the oldest one and the only one wild-born) was separated from the herd. Following her separation, the agonistic behaviour between the other rhinos significantly increased. In addition, play behaviour, especially between the male and females also increased. However, play behaviour is observed in adult male-female interactions in the wild only very rarely. We did not register any changes in socio-positive behaviour. Social dominance among the females, which might affect reproduction of subordinate animals, was not found. The presence of an old and experienced wild-born female in the herd during our study might have had a positive influence on the social interactions between other animals. Our results show that the composition of a white rhino group in captivity can have significant influence on the social interactions between the rhinos. Better knowledge of appropriate composition of white rhino groups in zoological gardens in terms of age, sex and wild or zoo origin might therefore improve animals’ well-being and also increase a chance for their reproduction.
Social and reproductive behaviour of critically endangered northern white rhinoceros (*Ceratotherium cottoni*) in a zoological garden

Ivana Cinková, Vítězslav Bičík

DEPARTMENT OF ZOOLOGY AND LAB OF ORNITHOLOGY, FACULTY OF SCIENCE, PALACKÝ UNIVERSITY, OLOMOUC, CZECH REPUBLIC, E-MAIL: IVANACINKOVA@CENTRUM.CZ
Introduction

- Northern white rhinos in captivity and in the wild
- Northern white rhinoceros – formerly subspecies of the white rhino (taxonomic revision: Groves, Fernando and Robovský, 2010)
- Low reproduction in captivity
- Behaviour:
  - Increased agonistic behaviour in captivity
  - Sociality
  - Dominance hierarchy?
Methods

- Zoological garden Dvůr Králové, 3 000 m² enclosure
- Breeding situation in the zoo
- Jul – Nov 2005
- Before our study started:
  - 3 adult females + 1 subadult female
  - + Adult female NESÁRÍ

1 month later

+ Adult bull SUNI

1 month later

Our study started
Methods

- Agonistic, cohesive (= socio-positive) and play behaviour

In the middle of our study, NESÁRÍ was separated from the group:

- Observations: before NESÁRÍ was separated (99 h 50 min) and after the separation (94 h 10 min)
- Scale scores to assess potential dominance hierarchy (following Jameson et al. 1999)
Results

- Behaviour before x after the separation (Wilcoxon paired test)

- After Bonferroni correction non-significant (for $P = 0.0125$)
Results

- Scale scores to assess potential dominance hierarchy
- 73% of all agonistic activities were directed towards the bull SUNI
Results

- Sociogram of cohesive interactions between the pairs of animals
Results: Reproduction

- Interest of the bull in females: 5x before NESÁRÍ was separated, 2x after her separation
- No mating occurred
Conclusion

- Separation of NESÁRÍ influenced the behaviour of other rhinos
- Increased agonistic behaviour $\rightarrow$ increased stress (Meister 1997) $\rightarrow$ lower chance for reproduction?
- Dominance hierarchy was not found
- NESÁRÍ (33 years, wild-born)
- Captive-born females are more likely to reproduce in the presence than in the absence of a wild-born female (Swaisgood et al. 2007)
- More studies on this topic are needed
Acknowledgements

- Zoo Dvůr Králové
- Dr. Kristina Tomášová
- Rhino keepers

The attendance at INTERNATIONAL ELEPHANT & RHINO CONSERVATION & RESEARCH SYMPOSIUM 2013 was supported by a grant from project PROVAZ (CZ.1.07/2.4.00/17.0138).

The results of this study were already published as:

Thank you for your attention
Management of a breeding herd of African elephants (*Loxodonta africana*) on a predominately forage diet.

Ray L Ball, DVM, Maura Middleton, BA
Tampa’s Lowry Park Zoo
Obligate forage feeding
Health and nutritional evaluation of gorillas on diets without commercial biscuits
Primates

Salad Days  Heart disease doesn't just hit humans... It's the leading killer among male zoo gorillas, and scientists want to know why. Obesity? Perhaps, but the term has yet to be defined for the primates. Diet? Likely, and Elana Heideloff Less of Cleveland Metroparks Zoo is trying to prove it. As part of a multi-year study, she's been feeding her two gorillas, Bibi and Mikhail (bottom), a doughnut menu meant to mimic the largely vegetarian one eaten in the wild. Healthy on baby greens, the new diet is also modeled after a heart-healthy human one, says Less. Judging by the 65 pounds each of her changes has shed so far, it's nothing to take lightly. —Graham Zuckerman

Old Menu
Since 2004, 22 gorillas at the zoo—in Cleveland and Columbus, Ohio, Milwaukee, North Carolina, Seattle, and Toronto—have been served a spiked diet, including beef, cheese, and other foods.

New Menu
The gorillas now instead eat many pounds a day of vegetables, fiber-rich products, including bananas, apples, and carrots. The new diet also includes the right amounts of the right nutrients that also are important human foods.

SKYCAST
Overhead this month in parts of the world:
August 19
Book of the Week: The Orange of Castile
August 22
Look, but don't Neglect...
Behavioral response of captive gorillas to
the introduction of a biscuit-free, high
fiber, low fruit diet

Richard A. Bergl¹, Ray Ball², Kristin Owen³, Sam Young³, Aaron Jesue¹, Courtni Bean¹, Barbara L. Sherman³, and Shana R. Lavin⁴

¹North Carolina Zoological Park
²Busch Gardens, Tampa Bay
³College of Veterinary Medicine, North Carolina State University
⁴Lincoln Park Zoo
White rhinoceroses

- Rotterdam 2011
- Serum electrolytes, minerals, vitamins
  - Zn deficiency in pregnancy
  - 9/10 births female
- Two facilities
Manatees

- Wild manatee rehabilitation
- Maintained on romaine lettuce
  - Small portion of native sea grasses
- 4 months to 2 years
African Elephants

• Landscape-scale feeding patterns of African elephant inferred from carbon isotope analysis of feces.

• Predominately grazers

• Opportunistic browsers

• Tolerant of foods with relatively low nutritional value
Concerns with concentrates

• Low fiber
  – peNDF

• High starch
  – CHO metabolism
  – Obesity
  – Inflammatory?

• Reduced feeding times
  – Stereotypy
  – Altered mineral physiology
Can elephants in managed care be fed predominately forages?

<table>
<thead>
<tr>
<th>Current Concerns</th>
<th>Objectives</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Obesity</td>
<td>• Leaner animals</td>
</tr>
<tr>
<td>• Foot concerns</td>
<td>• Improve foot health</td>
</tr>
<tr>
<td>• Reproduction limitations</td>
<td>• Improve reproduction</td>
</tr>
<tr>
<td>• Inflammatory conditions</td>
<td>• Reduce inflammatory stress</td>
</tr>
<tr>
<td>• Secondary infectious Dz</td>
<td>• Reduce secondary infections</td>
</tr>
<tr>
<td>– Tuberculosis</td>
<td></td>
</tr>
<tr>
<td>– Salmonella</td>
<td></td>
</tr>
<tr>
<td>– EEHV</td>
<td></td>
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</table>
Can elephants in managed care be fed predominately forages?

• Safe
• Provides adequate nutrients
• Palatable
• Promotes health
• Supports reproduction
Diet Comparison

Concentrate based
- 30kgs grass hay
- 4.5kg elephant supplement
- 2kg oats
- 4kg sweet feed
- Bread enrichment
- Produce training
- Browse enrichment

Forage based
- Grass hay ad libitum
  - Coastal
- 2kgs alfalfa hay
- 3kgs produce training
- Browse daily
Browse

Laurel oak (*Quercus hemisphaerica*)
Live oak (*Quercus virginiana*)
<table>
<thead>
<tr>
<th></th>
<th>Elephant Supplement</th>
<th></th>
<th>Oak</th>
<th></th>
<th></th>
<th>Hay</th>
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<tbody>
<tr>
<td></td>
<td>As Fed</td>
<td>DM</td>
<td>As Fed</td>
<td>DM</td>
<td>As Fed</td>
<td>DM</td>
<td></td>
</tr>
<tr>
<td>% Moisture</td>
<td>12.0</td>
<td></td>
<td>50.2</td>
<td></td>
<td>8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Dry Matter</td>
<td>88.0</td>
<td></td>
<td>49.8</td>
<td></td>
<td>91.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Crude Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21.3</td>
<td>4.9</td>
</tr>
<tr>
<td>% ADF</td>
<td>12.9</td>
<td>14.6</td>
<td>23.9</td>
<td>48</td>
<td>38.1</td>
<td>41.6</td>
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<tr>
<td>% NDF</td>
<td>24.6</td>
<td>27.9</td>
<td>25.8</td>
<td>51.8</td>
<td>60.3</td>
<td>66.0</td>
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<tr>
<td>% Lignin</td>
<td>4.0</td>
<td>4.6</td>
<td>9.4</td>
<td>18.9</td>
<td>5.7</td>
<td>6.3</td>
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</tr>
<tr>
<td>% Starch</td>
<td>11.4</td>
<td>12.9</td>
<td>0.1</td>
<td>0.1</td>
<td>0.7</td>
<td>0.7</td>
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</tr>
<tr>
<td>% Sugar</td>
<td>7.9</td>
<td>8.9</td>
<td>4.2</td>
<td>8.3</td>
<td>8.7</td>
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<tr>
<td>% Crude Fat</td>
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<td>4.8</td>
<td>1.4</td>
<td>2.9</td>
<td>2.3</td>
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<td>% Ash</td>
<td>10.73</td>
<td>12.20</td>
<td>2.06</td>
<td>4.14</td>
<td>6.76</td>
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<td>1.67</td>
<td>0.29</td>
<td>0.58</td>
<td>.24</td>
<td>.26</td>
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<tr>
<td>% Phosphorus</td>
<td>.83</td>
<td>.94</td>
<td>0.07</td>
<td>0.14</td>
<td>.18</td>
<td>.20</td>
<td></td>
</tr>
<tr>
<td>% Magnesium</td>
<td>.27</td>
<td>.31</td>
<td>0.07</td>
<td>0.14</td>
<td>.11</td>
<td>.12</td>
<td></td>
</tr>
<tr>
<td>% Potassium</td>
<td>1.81</td>
<td>2.06</td>
<td>0.06</td>
<td>0.12</td>
<td>2.23</td>
<td>2.44</td>
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<tr>
<td>% Sodium</td>
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<td>1.141</td>
<td>0.45</td>
<td>0.9</td>
<td>.003</td>
<td>.003</td>
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<tr>
<td>PPM Iron</td>
<td>611</td>
<td>695</td>
<td>0.01</td>
<td>0.02</td>
<td>74</td>
<td>81</td>
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<tr>
<td>PPM Zinc</td>
<td>347</td>
<td>394</td>
<td>84</td>
<td>168</td>
<td>18</td>
<td>20</td>
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<td>PPM Copper</td>
<td>51</td>
<td>57</td>
<td>11</td>
<td>23</td>
<td>9</td>
<td>10</td>
<td></td>
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<td>PPM Manganese</td>
<td>229</td>
<td>260</td>
<td>4</td>
<td>7</td>
<td>57</td>
<td>62</td>
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<tr>
<td>PPM Molybdenum</td>
<td>1.9</td>
<td>2.1</td>
<td>8</td>
<td>16</td>
<td>0.5</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>
Comparisons on diets

• 1.3 African elephants
  – Imported
  – 1.2 Swaziland
  – Clinically healthy
  – Trunk wash negative

• Body weights/condition

• Reproduction

• Bloods

• Blood
  – Hematology
  – Serum biochemistry
  – Vitamin E
  – Vitamin D
  – Micro minerals
Hematology

Concentrate
- WBC $11.79 \times 10^3/\mu l$
  - SD 2.799, N=25
- Monocytes $4.599 \times 10^3/\mu l$
  - SD 2.456, N=25

Forage
- WBC $14.17 \times 10^3/\mu l$
  - SD 2.484, N=54
- Monocytes $2.569 \times 10^3/\mu l$
  - SD 2.067, N=54
Hematology

Concentrate
- Hematocrit 36.02 %
  - SD 2.783, N= 24
- Hemoglobin 12.72 g/dl
  - SD 1.512, N= 25
- RBC 2.882 *10^6/ul
  - SD 0.416, N=25
- Platelets 514 *10^3/ul
  - SD 223.7, N=24

Forage
- Hematocrit 33.63 %
  - SD 4.016, N= 56
- Hemoglobin 11.46 g/dl
  - SD 1.138, N= 53
- RBC 2.724 *10^6/ul
  - SD 0.255, N=53
- Platelets 200.5*10^3/ul
  - SD 188, N=41
Hematology Differences

• Monocytes
  – Antigen stimulus
  – Careful not to over interpret
• Hct
  – Hydration status
• Platelets
  – Chronic inflammation

• Lower Hct seen in white rhinos fed forage only
  – Ball et al IEF/IRF Rotterdam 2011
• Concentrate fed elephants hemoconcentrating??
  • Pairs with serum protein
Serum Biochemistry

Concentrate

- Total protein 7.632 g/dl
  - SD 0.34, N=25
- Albumin 3.242 g/dl
  - SD 0.262, N=24
- Globulin 4.388 g/dl
  - SD 0.37, N=24

Forage

- Total protein 6.894 g/dl
  - SD 0.83, N=16
- Albumin 2.85 g/dl
  - SD 0.434, N=16
- Globulin 3.929 g/dl
  - SD 0.658, N=17
## Serum Biochemistry

**Concentrate**
- Creatinine 1.356 mg/dl  
  - SD 0.25, N=24
- BUN 8.44 mg/dl  
  - SD 3.097, N=25
- Amylase 9924 SU  
  - SD 2707, N=23
- Triglyceride 27.83 mg/dl  
  - SD 11.05, N=23

**Forage**
- Creatinine 1.1 mg/dl  
  - SD 0.181, N=16
- BUN 6.5 mg/dl  
  - SD 2.875, N=16
- Amylase 7225 SU  
  - SD 4076, N=16
- Triglyceride 22.38 mg/dl  
  - SD 3.204, N=54
Serum Biochemistry

• BUN, Cr lower in forage fed animals
  – Lower protein intake
  – Improvement in renal function?
    • Subclinical renal insufficiency
• Amylase
  – Renal disease may prolong clearance

• Lower protein intake still appears adequate
  – Reproduction
  – Lactation
  – Calf growth
• Concentrate fed elephants under some “renal stress”??
  – Contribute to low Vit D?
Vitamin D (25-OH, MSU)

• No values before diet change
• MSU mean on 86 individuals 14mmol/L
• Forage fed ranged from 20 to 27 mmol/L
  – N=4
Descriptive epidemiology using serology in an outbreak of *Mycobacterium tuberculosis* in managed Asian elephants (*Elephas maximus*) at a single facility.

Ray L. Ball, DVM, Lowry Park Zoo, Tampa, Florida, USA,
Sumeet Gupta, Vet Student Scholar, Ohio State University

Vitamin D Levels in Captive U.S. Elephants

Asian elephants from different zoos in the United States.

compared across geographic location to diet and blood vitamin D values of elephants from Thailand

Asian elephants **in northern latitudes of the United States** exhibit lower concentrations of blood vitamin D than those in southern latitudes and those living in their natural habitat of southeast Asia.
TUBERCULOSIS

Reactivation of tuberculosis and vitamin D deficiency: the contribution of diet and exposure to sunlight
A Sita-Lumsden, G Lapthorn, R Swaminathan, H J Milburn


- Suggest abnormal handling of this vitamin
- Renal pathology
- Vitamin mal-absorption
- Granulomatous disease
Decreased synthesis of 1,25-dihydroxyvitamin D

Chronic kidney disease

- Stages 2 and 3 (estimated glomerular filtration rate, 31 to 89 ml/min/1.73 m²)
- Hyperphosphatemia increases fibroblast growth factor 23, which decreases 25-hydroxyvitamin D-1α-hydroxylase activity

Causes decreased fractional excretion of phosphorus and decreased serum levels of 1,25-dihydroxyvitamin D

- Stages 4 and 5 (estimated glomerular filtration rate <30 ml/min/1.73 m²)
- Inability to produce adequate amounts of 1,25-dihydroxyvitamin D

Causes hypocalcemia, secondary hyperparathyroidism, and renal bone disease

---

Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis

Kelechi E Nnoaham¹* and Aileen Clarke²
What is the most important nutrient?

– Water

• Water comprises 99% of all molecules within the animal’s body
  – Neonate bird or mammal (71-88% of BW)
  – Decreases as they grow
• Decreased in obese animals (50-65%)
  – Muscle 72%
  – Fat 3–7%
Vitamin E (MSU)

Concentrates
• Mean 0.35 ug/ml
  – N=2

Forage
• Mean 1.39 ug/ml
  – N=4

• Free ranging values reported 0.41 to 0.61 ug/ml
• Same imported group at another facility
• 0.41ug/ml to 0.13ug/ml in 4 years
  • Schlegel et al 2008
• 307 samples from two other Florida facilities
  • Mean 0.618 ug/ml
Vitamin E antioxidant

• More fresh browse
  – Better Vit E intake
• Less inflammation
  – Lower platelets
  – Lower globulins
  – Lower monocytes
• Less oxidative stress = higher levels of Vit E??
Minerals
all statistically same

- Cobalt ng/ml
- Copper ug/ml
- Iron ug/dl
- Mang ng/ml
- Molyb ng/ml
- Zinc ug/ml
- Selenium ng/ml

- P=0.06
- 78 Conc; 75 Forage
- 1.25 Conc; 1.172 Forage
- 67 Conc; 54.8 Forage
MATJEKA

Weight (lbs.)

DATE

Forage feeding

• Appears to be safe
• Sustainable
• Meets basic needs
• Meets needs for reproduction
  – Lactation
  – Calf development

• Trivers-Willard hypothesis, maternal condition at or around conception affects the secondary sex ratio in mammals
• White rhinos
• African elephants
  – Swaziland imports
Forage feeding

- May alter hydration status
- May improve renal health
- May be less inflammatory

- Improve hydration
- Improve renal health
  - Improves Vit D
Further analysis

- **Fatty acids**
  - Strengthened digital cushion
  - Data in cattle
  - The fatty acid content of the bovine digital pad can be influenced by lipid intake (Raber, M., M., 2006)
  - Fatty acids have been compared in African elephants between wild animals and managed ones fed concentrates (Clauss 2006).
Further analysis

• Heat stress and diet
  – Forages produce more heat
• Obesity
• Sex selection in offspring
  – Trivers-Willard hypothesis
Acknowledgements

- TLPZ Elephant Staff
- TLPZ Veterinary Staff
- City of Tampa
- Oscar Nurse Landscaping
Asian Elephant Support (AES) is a U.S. non-profit foundation dedicated to the care and conservation of elephants in Asian range countries, and to the people whose lives are intertwined with this magnificent and endangered species.

www.asianelephantsupport.org
Mission of Asian Elephant Support

- Provide financial support for elephant projects in Asian range countries that meet our criteria for care of captive elephants and for conservation of the species.
- Increase awareness of the needs and future of the Asian elephant.
- Increase awareness of the humane treatment of elephants living in captivity.
- Provide educational opportunities to those persons who care for elephants in Asian range countries.
Board of Directors
Hoof knives for Mahouts–India

• Collaboration with EMA Conservation Committee

• Raised $1755.52 for mahouts in Assam, India
ElefantAsia
Laos PDR

Portable scales donated by AES
Field Course in Emerging Diseases of Asian Elephants

- Follow up field course to Regional Asian Elephant Veterinary Workshop
- Funded by a grant from the USFWS’s Asian Elephant Conservation Fund
- Kerala, India – November 2012

- Hosted by the Kerala Veterinary and Animal Sciences University
Elephant Endotheliotropic Herpesvirus (EEHV)

- In conjunction with a grant from USFWS’s Asian Elephant Conservation Fund
- Confirmed first two cases of EEHV in Sumatra, Indonesia
Myanmar

- Facilitated a visit by veterinarians – hosted by Myanmar Timber Enterprise
- Provided funding for elephant medicine
AES provides emergency funding for rescued orphans and other individual elephants needing immediate assistance.
AGAM

- Found in an abandoned well in Aceh, Sumatra
- Villagers contacted VESSWIC
- Unable to locate herd so relocated to EEC in Saree, Sumatra.
RAJU

- Orphaned possibly due to HEC issue
- Weak and dehydrated
- Under one month old
Documenting Indigenous Traditional Knowledge of the Asian Elephant in Captivity

Asian Nature Conservation Foundation
Centre for Ecological Sciences
Indian Institute of Science
Bangalore, India

- Record the relationship between the elephant and its keeper
- Provide valuable insight into the mitigation of human–elephant conflict issues
2nd Regional Asian Elephant Veterinary Workshop, Myanmar 2014

Funded by a grant from the USFWS Asian Elephant Conservation Fund
AES Activities
Fundraisers

California Pizza Kitchen
3rd Annual Fundraiser
E-cards

Happy Holidays

Father’s Day

Happy Birthday

Happy Mother’s Day
Elephant Education

1st Grade Presentation
Asian Festival – May 2013

Earth Day at the Virginia Zoo – April 2013
Ways to Help

- Donations
  Mail or PayPal on line

- Good Search

- Good Dining

- Fundraisers
  California Pizza Kitchen
  Buffalo Exchange
For More Information

Website
www.asianelephantsupport.org

E-mail
info@asianelephantsupport.org

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POSTERS
POSTERS

**Allomothering Among Captive African elephants**  
Laura Beer, Heather R. Bates and Robert H. I. Dale  
*Butler University*

According to P.C. Lee (1987), interactions between African elephant calves and other elephants are frequent, consisting of friendly, relaxed interactions or of adults providing assistance to calves. When a female elephant other than its mother provides comfort, care or protection a calf, it is called allomothering. Using video recordings of African elephants at the Indianapolis Zoo, we compared the interactions between several calves and Sophi (the “matriarch”/dominant female) and Tombi (a relatively subordinate adult in the group). Note that allomothering may occur either after an adult approaches a calf or after a calf approaches an adult. We examined the nature of the interactions between the calves and the adults.

**Rhinoceros Horns and Imitations in the Trade**  
Vanessa Blount, B.C. Yates, and E.O. Espinoza  
*National Fish and Wildlife Forensics Laboratory*

The illegal trade in rhino horn is a leading contributing factor to the extinction of rhinoceros. Former evidentiary material can be used to further our knowledge base on how the horns are removed, processed, shipped, and modified for sale as whole horns or artifacts. Mechanisms of fakery can be detected using variable light source and low magnification to show how horse hooves and cattle horns are made into rhino horn imitations. Additionally, illegal fakes have been found made from carved elephant toenail. The implications of selling fake rhino horn include continued promulgation of the desire for rhino products and wasted time agencies/organizations spend in monitoring alleged contraband.

**Elephants for Africa: Conservation through Research and Education**  
**Preliminary Findings on Male Elephant Sociality in the Makgadikgadi National Park, Botswana**  
Kate Evans, Mphoeng Ofithile, and Miguel Cases  
*Elephants for Africa*

Elephants for Africa’s work focuses on male elephants, a little understood aspect of elephant ecology. Studying the elephants that are utilising the Makgadikgadi National Park (MNP), historically a peripheral elephant range, will educate us on the conservation of elephant landscapes in order to increase range and connectivity between populations; a conservation priority of the World Wildlife Fund for Nature (WWF). The MNP as part of the Northern Conservation Area, is home to the largest remaining population of elephant in the world and is part of the Kavango-Zambezi Transfrontier Conservation Area (KAZA), which has been highlighted a priority area for the conservation of the African elephant by the WWF.

In recent years there has been an influx of male elephants into this area with the current population being made up predominantly of bulls with sightings of male groups more than 100 strong socialising...
together (Bradley, pers comms). Male groups as large as these have not been sighted in Africa in recent history, giving us a unique opportunity to further understand the social ecology of male elephants.

Understanding the population dynamics of the elephants utilising the area and potential fluctuations in the population alongside habitat changes; contributing to both the national (Department of Environmental Affairs 2008), regional (Department of Environmental Affairs and Centre for Applied Research 2010) and the African Elephant Specialist Group (AfESG) research priorities. The Okavango Delta is extremely dynamic, relying on a flood pulsing system, which shifts the wet and dry seasons, since 2008 the flood levels have increased dramatically, through necessity, the elephants are periodically forced into new and old ranges. This along with a recent increase and then stabilization of the elephant population in Northern Botswana (Chase 2010) may have caused the recent influx of male elephants into the MNP and surrounding areas (Department of Environmental Affairs and Centre for Applied Research 2010).

Initial findings show that groups as large as 52 male elephants are meeting at the Boteti River not only to drink and mudbath but to socialize. Leading us to wonder what role these large social groupings have in male elephant sociality and the implications for their conservation and welfare both in-situ and ex-situ. Comparing data from our 10-yaer database in the Okavango Delta we will present preliminary findings on the differences in male social ecology between these two predominantly male elephant areas in Botswana.

Differentiation of Mycobacterium Species from Elephant Respiratory Samples in Nepal, Using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) Analysis

Corissa Miller¹, Kristin Warren², Carly Holyoake², Ian Robertson², Gretchen Kaufman³, Tierra Evans⁴, Dibesh Karmacharya⁵, Sulochana Manandhar⁵, Kshitiz Shrestha⁵ and Maheshwar Dhakal⁶

¹Ecotone Wildlife Veterinary Services, Australia; ²School of Veterinary and Biomedical Sciences, Murdoch University, Australia; ³Paul G. Allen School for Global Animal Health, Washington State University, USA; ⁴Center for Conservation Medicine, Tufts Cummings School of Veterinary Medicine, Tufts University, USA; ⁵Center for Molecular Dynamics Nepal (CMDN), Nepal; ⁶Department of National Parks and Wildlife Conservation, Government of Nepal

Tuberculosis caused by Mycobacterium tuberculosis complex (MTBC) species poses a significant threat to the health and welfare of Asian elephants, and to the conservation of the species as a whole (Greenwald et al. 2009). Both Mycobacterium tuberculosis (M. tb) and Mycobacterium bovis (M. bovis) are endemic to Nepal, yet it is unknown which species are responsible for pulmonary mycobacteriosis in Nepal’s elephant population (NETCMAP 2011). Captive elephants interact closely with humans, wildlife and domestic species, increasing the risk for bidirectional transmission of Mycobacterium species and raising important public health, economic and conservation concerns (Murphree et al. 2011; Oh et al. 2002). It is widely accepted that, while early detection and management are essential for successful disease control in elephants, key inadequacies exist in current tuberculosis diagnostics (Angkawanish et al. 2010; Montali et al. 1998). Diagnosis relies on trunk wash (TW) culture and serology (Mikota and Maslow 2011). However, TW culture is impractical in many countries and lacks sensitivity (Mikota et al. 2006). Serology, while sensitive, does not differentiate the various Mycobacterium species, nor is it useful for monitoring elephants post-treatment (Kay et al 2010). The objective outcome of this research was to minimise the impact of tuberculosis on Asian
elephant populations, through development of a novel molecular diagnostic technique, using Nepal’s captive elephant population as a model.

A gyrB-based PCR-RFLP assay for the detection of MTBC species was developed, building on preliminary work by Wilson and others (2008) at the Center of Molecular Dynamics Nepal (CMDN). The technique was found to be capable of detecting and differentiating Mycobacterium tuberculosis and Mycobacterium bovis DNA from elephant trunk wash and nasal drip samples. Preliminary genetic sequencing confirmed potential for future application for identification of drug resistance. Despite multiple limitations in relation to study protocol, field sampling and in-country laboratory and technical capabilities, it was demonstrated that the PCR-RFLP technique is practical, accessible and relevant to developing Asian elephant range countries such as Nepal, and provides a potentially valuable addition to the current array of diagnostic options. The findings and limitations of this research were collated to provide recommendations for future research and improved management strategies, to minimise the impact of tuberculosis on the wild and captive populations of this endangered species.

References


**Preliminary Recordings of Wild Asian Elephant (Elephas Maximus) Vocalizations in Preparation for Playback Experiments**

Michael A. Pardo¹*, Shermin de Silva, PhD²,³

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Communication complexity is thought to increase with social complexity. Asian elephants live in a fission-fusion society and form multiple long-term social relationships, suggesting that their communication system may be particularly complex. They produce at least fourteen distinct call types¹, but the meanings of these calls have never been experimentally determined. Growls (a type of low-frequency harmonic call) are individually distinct, but also highly variable within a given individual¹. However, it is not known whether there are different structural or functional categories of growls. Combination calls are composed of a broadband segment immediately followed by a low-frequency harmonic segment¹. Anecdotal reports suggest that elephants may respond more to combination calls than to low-frequency calls alone. The broadband segment may help call attention to the information contained in the low-frequency segment. Alternatively, the low-frequency segment may allow individual identity to be assigned to a broadband call. In December 2012 and January 2013, we recorded fifty-three calls from wild Asian elephants in Uda Walawe National Park, Sri Lanka, and categorized the vocalizations into basic call types according to de Silva¹ by visual inspection of the spectrograms. Thirty-one calls were low-frequency signals with clear harmonics (growls and rumbles), eleven were broadband signals with no clear harmonics (barks, roars, and longroars), and five were combination calls (bark-rumbles). The remainder of the calls consisted of higher frequency sounds (trumpets and squeaks). We will record additional calls and pool our recordings with a pre-existing call library recorded by the Uda Walawe Elephant Research Project. We will then use cluster and discriminant function analysis to determine if growls can be subdivided into discreet structural categories. If structural categories exist, we will use playback experiments to test whether they correspond to functional categories. We will also use playback experiments to test multiple hypotheses for the function of combination calls.
ACKNOWLEDGMENTS
We are deeply grateful to the staff of the Uda Walawe Elephant Research Project, without whom this research would not be possible. We also thank the Sri Lankan Department of Wildlife Conservation for permission to work in Uda Walawe National Park. This project was funded by grants from the Cornell University Graduate School and the Athena Fund of the Cornell Lab of Ornithology.

LITERATURE CITED

Confirmation of the First Case of Endotheliotrophic Elephant Herpes Virus (EEHV) Infection in Nepal Using a Real-Time Quantitative PCR Assay and DNA Sequence Analysis
Suraj Subedi¹, Corissa Miller², Dibesh Karmacharya³ and Sulochana Manandhar³
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Elephant endotheliotropic herpes virus (EEHV) presents a growing threat to the health and conservation of both captive and wild endangered Asian elephant populations worldwide (Cracknell 2008). In the acute form the disease is characterized by a sudden onset of lethargy, oedema of the head, proboscis and limbs, oral ulcers, and diffuse internal haemorrhaging (Garner et al 2009; Miller and Fowler 2012). In acute cases, death often results within one week following the onset of symptoms, and few cases have survived with intensive treatment (Hayward 2012). There is currently limited knowledge of the prevalence of EEHV in Nepal, a country with both captive and wild Asian elephant populations. However, since 2008, four elephant calves have succumbed to unconfirmed but presumed herpes virus infections (Gairhe 2012). Findings from a study of the Chitwan National Park (CNP) elephant breeding center in mid-2012 suggest the presence of latent EEHV infections in a number of adults within the breeding herd, although PCR and sequencing results were inconclusive (Kaufman et al, unpublished). In December 2012 a juvenile female elephant from the CNP breeding herd presented with ante-mortem and post-mortem changes characteristic of acute EEHV. Conjunctival swabs and heart, liver, kidney and blood samples were collected by the National Trust for Nature Conservation (NTNC) and analysed at the Center for Molecular Dynamics Nepal (CMDN). Samples were analysed using a validated real-time quantitative PCR assay (Hardman et al 2010; Latimer et al 2011), and all samples confirmed to be positive for EEHV. Using DNA sequencing and viral gene sub-typing analysis (Stanton et al 2010; Stanton et al 2012, in review) all samples were subsequently identified as subtype EEHV1A. These results mark the first PCR-confirmed case of EEHV infection in Nepal, and demonstrate the presence of EEHV in the CNP captive elephant population. Such findings support the hypothesis that latently infected individuals reside within the CNP breeding herd, and highlight the urgent need for adaptive management to minimise morbidity and mortality. Further research into EEHV prevalence, epidemiology and dynamics in Nepal are recommended in order to mitigate the impact of this disease on both captive and wild Asian elephant populations.

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Baby Steps
Kathy Suthard and Diane Hagey
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The Rhino Keepers Association
Jane Kennedy
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Education Outreach Materials from USFWS and Asian Elephant Conservation Fund
Mini Nagendran
Asian Elephant Conservation Fund and U.S. Fish and wildlife Service
Allomothering among Captive African Elephants
Laura Beer, Heather R. Bates, Robert H. I. Dale
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Abstract
We observed four elephants in a large outdoor exhibit at the Indianapolis Zoo (see diagram) over three sessions for a total of minutes. During each session, a female calf, Nyah (3 months), her mother, Ivory (30 years), her sister, Zahara (6 years) and the group’s “matriarch”, Sophi (44 years – the oldest, largest elephant), were present.

We counted the number of times that Nyah was within one adult body length of another elephant (about 3m), which elephant initiated the interaction, and the duration of each interaction. Of course, it was possible for Nyah to be near more than one elephant at the same time.

Introduction
It is clear that allomothering (Lee, 1987) is common among African elephants. It is less clear which elephant initiates or terminates contact in an allomothering interaction, or how allomothering is distributed within a group of captive elephants. This study addresses these two questions.

Results

The Elephant Exhibit

Nyah: Interaction Times (s)

<table>
<thead>
<tr>
<th>Status</th>
<th>Duration(s)</th>
</tr>
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<tbody>
<tr>
<td>Not visible</td>
<td>84</td>
</tr>
<tr>
<td>Alone</td>
<td>1372</td>
</tr>
<tr>
<td>I</td>
<td>538</td>
</tr>
<tr>
<td>S</td>
<td>952</td>
</tr>
<tr>
<td>Z</td>
<td>805</td>
</tr>
<tr>
<td>IS</td>
<td>464</td>
</tr>
<tr>
<td>IZ</td>
<td>288</td>
</tr>
<tr>
<td>SZ</td>
<td>691</td>
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<tr>
<td>ISZ</td>
<td>260</td>
</tr>
<tr>
<td>Total</td>
<td>5752</td>
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Nyah: Time with each elephant

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<th>Time (s)</th>
<th>p (Visible time)</th>
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<tr>
<td>Alone</td>
<td>1372</td>
<td>0.24</td>
</tr>
<tr>
<td>With Ivory</td>
<td>1549</td>
<td>0.27</td>
</tr>
<tr>
<td>With Sophi</td>
<td>2666</td>
<td>0.47</td>
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<td>With Zahara</td>
<td>2343</td>
<td>0.41</td>
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<tr>
<td>Visible</td>
<td>5668</td>
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Discussion
Nyah spent about one quarter of her time either alone or with her mother, and nearly half of her time near her sister or the matriarch of the group. She spent a majority of her time near two or more of the other elephant. This is what we had expected to happen based on previous data from other locations.

Each elephant initiated contacts with Nyah at about the same frequency with which it terminated contacts with her. However, Nyah interacted differently with each of the other elephants. She had more separate contacts with Zahara than with either of the other elephants, reflecting the higher activity levels of the two calves, \( \chi^2 (1) = 7.1, p < 0.01 \) [Zahara vs. the other two elephants combined].

Nyah was more likely to initiate contacts with Ivory and Sophi than vice versa, \( p < 0.01 \) in each case. Sophi, in particular, rarely initiated contacts with Nyah. On the other hand, Zahara was more likely to initiate contacts with Nyah, than vice versa, \( p < 0.001 \).

It appears that Nyah sought out Ivory and, especially, Sophi, whereas Zahara was more likely to seek out Nyah.

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Lee, P. C., & Moss, C. J. Calf development and maternal rearing strategies (pp. 224-237).
2013 International Elephant & Rhino Conservation and Research Symposium

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**Butler Gates, LLC Goal:**
To provide a new solution for lightweight, extremely strong gates made out of Carbon Fiber. These gates are designed for maximum safety and minimal maintenance. They move fast, especially in emergency situations. All the equipment is concealed for safety and is completely "Elephant Proof". Gates have a wireless safety edge, which will reverse operation upon activation. There are no ground tracks to clean, which aids in the fast and smooth operation of the gate. The gate system can be integrated with Hardscape Technologies Inc. High Strength Patented Modular Fencing System. Every Gate System is assembled, run, and tested prior to being shipped to a site. Systems run on DC power, tied to line voltage with a DC inverter and battery bank, with optional solar panels for remote locations.

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To provide a patented crash resistant fence that is pre-built in modular units that can be shipped anywhere in the world and requires only a two foot deep footing. 12” vertical steel posts are welded into each of the steel fence units. Completed modular units installation is a simple 3-step process. Fence units are placed in the excavated trench, bolted together and filled in with concrete or concrete blocks. The modular fence units come primed as a base model with options of being primed and painted steel, or galvanized steel or stainless steel. Steel cable is integrated with the modular system after installation with connectors for multiple runs of cable. The cable is electrified with line voltage with a DC inverter to provide 24V power. Optional solar panels with a DC battery bank are available for remote locations.

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INTERNATIONAL CONSERVATION CENTER
International Conservation Center

a conservation, education & breeding center
742 acres in Somerset County, PA
2 miles of interior fencing
Spring fed frost free waterers and run-in sheds
5 miles of electrified perimeter fencing 10 ft high
Maintenance facility with equipment (65 x 165 ft.)
Grain holding & mixing equipment
12,000 bale hay barn
Phase I
Elephant Care Facility

$3 million
10,816 sq. ft.
Day Stall = ~4500 sq. ft.

Two stalls 1,200 sq. ft.
One stall 1,100 sq. ft.
Cabin project
Camp Program
Additional Species
2009 Elephant TAG – SSP Meeting
3rd Annual Management and Research Priorities of Tuberculosis for Elephants in Human Care Workshop Proceedings

August 25-26th, 2013
Pittsburgh, PA, USA.

Hosted by:
American Association of Zoo Veterinarians
Association of Zoos and Aquariums Elephant Taxon Advisory Group
Elephant Managers Association
International Elephant Foundation
Ringling Bros. Center for Elephant Conservation
Pittsburgh Zoo
The 3rd Annual ECT met in Pittsburgh in conjunction with the 2013 International Elephant and Rhino Conservation and Research Symposium sponsored by the International Elephant Foundation (IEF), the International Rhino Foundation and the Pittsburgh Zoo and PPG Aquarium. A total of 45 people attended the ECT representing expertise in elephant husbandry, veterinary medicine, human health, public health, infectious disease and animal science.

**Sunday, 25 August 2013**

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**08:00 – 10:15 Session I**

08:00 – 08:15  Welcome and Introductions  
Previous workshop report

08:15 – 08:30  Differentiation of *Mycobacterium* species from Asian elephant (*Elephas maximus*) respiratory samples in Nepal, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis  
Corissa Miller

08:30 – 08:45  Tuberculosis Infection in Wild and Captive Asian Elephants in Southern India  
David Abraham

08:45 – 09:00  TB and elephants in Asia  
Christopher Stremme

09:00 – 09:15  TB management: What should never happen!  
Francis Olivet-Courtois

09:15 – 09:30  Nepal Elephant Healthcare and TB Surveillance program  
Hank Hammatt

09:30 – 09:45  Elephant trunk wash procedure  
Mike McClure

9:45 – 10:00  Testing for tuberculosis in elephants: what is the evidence?  
David Miller

10:00 – 10:15  Point prevalence and incidence of *Mycobacterium tuberculosis* complex in captive elephants in the United States  
Ramiro Isaza

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**10:15 – 10:30 Break**

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**10:30 – 12:10 Session II**

10:30 – 10:45  Alphabet Soup: MICs, PK, BP, mTB and Elephants  
Rob Hunter

10:45 – 11:00  Factors Associated with TB Transmission in Humans: Relevance in the Elephant Population
Jennifer Furin
11:00 – 11:15  Elephants, TB and Research
Michele Miller

11:15 – 12:30  Introduction to Workshop Procedures
Discussion and identification of management and research priorities for
working groups
Jill Allread - Facilitator

12:30 – 13:30  Lunch

13:30 – 15:30  Session III - Plenary
Jill Allread - Facilitator

15:30 – 15:45  Break

15:45 – 17:30  Session IV – Working Groups
Jill Allread - Facilitator

Monday, 26 August 2013

08:00 – 10:15  Session V - Working Groups
Jill Allread - Facilitator

10:15 – 10:30  Break

10:30 – 12:00  Session VI - Plenary
Jill Allread - Facilitator

12:00 – 13:00  Lunch

13:00 – 15:00  Session VII – Working Groups
Jill Allread - Facilitator

15:00 – 15:15  Break

15:15 – 17:00  Session VIII - Plenary
Jill Allread - Facilitator
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<td>Have Trunk Will Travel</td>
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Since 1998, the United States Department of Agriculture – Animal and Plant Health Inspection Service Animal Care (USDA-APHIS-AC) has relied upon a series of updated guidelines for the management of tuberculosis in elephants that were developed by ad hoc committees (under the auspices of the American Association of Zoo Veterinarians) of veterinarians, researchers and epidemiologists concerned with the management of *Mycobacterium tuberculosis* (MTB) complex in elephants. Since 1998, a trunk wash or post mortem culture positive for MTB was determined to be the only way to identify an elephant with an active infection and each of the guidelines relied exclusively on this diagnostic test. In 2007, the Elephant Tuberculosis Advisory Subcommittee of the United States Animal Health Association’s (USAHA) Committee on Tuberculosis was established to take responsibility for developing Guidelines for the Control of Tuberculosis in Elephants (Elephant TB Guidelines). In 2008, the USDA Elephant TB Guidelines incorporated the use of a USDA licensed serological screening test (ElephantTB STAT-PAK®, Chembio Diagnostics Inc., New Medford, NY) for TB antibodies (*M. tb* and *M. bovis*) as a complement to culture to identify “infected” elephants, and elephants at risk of developing tuberculosis or shedding organisms. In 2010, USDA–APHIS-AC incorporated these serological tests into its elephant tuberculosis regulatory program. In 2010 the USAHA subcommittee recommended changes to the Guidelines that placed greater emphasis on the ElephantTB STAT-PAK® and a test (the Multi-Antigen Print Immunoassay (MAPIA)) that was proposed to be confirmatory. STAT-PAK and MAPIA results were proposed as the basis for establishing elephant tuberculosis status categories, together with exposure history, but these categories were not rigorously established using the full state of scientific knowledge. Although the 2010 Guidelines established restrictions based upon test results there are differences of opinion about the interpretation of serologic tests results.

On April 5-6, 2011, USDA-APHIS-AC held a seminar entitled Tuberculosis in Elephants: Science, Myths, and Beyond! in Kansas City, Missouri. This scientific meeting consisted of presentations from contributors with a range of backgrounds in human and veterinary medicine, regulatory and public health infectious disease, epidemiologists, tuberculosis researchers, and a specialist in risk evaluation. Although the presentations and following discussions were elucidating, many in attendance identified gaps in existing scientific knowledge. These gaps call into question the basis for decision making and guideline development. In addition, it is believed by many that greater stakeholder involvement in the process of developing future Elephant TB Guidelines is needed to address concerns about the impact on all parties and to meet current standards for the development of evidence-based policy.

Dr. Chester Gipson, Deputy Administrator of Animal Care at USDA, commented at the end of the workshop that the ongoing development of the Guidelines for the Control of Elephant Tuberculosis is an open and transparent process and he encouraged the stakeholders to become more involved in the discussion and to provide scientific information on which to base future guidelines. To that end, the American Association of Zoo Veterinarians, Association of Zoos and Aquariums Elephant Taxon Advisory Group, Elephant Managers Association, Fort Worth Zoo, International Elephant Foundation, and Ringling Bros. Center for Elephant Conservation formed a partnership to organize and facilitate the first annual workshop “Management and Research Priorities of Tuberculosis for Elephants in Human Care - Stakeholders Task Force” to
share current scientific research and experimental information about tuberculosis in elephants. This was accomplished through facilitated and breakout sessions to identify research and management priorities and develop action plans to further the understanding of the identification, management and treatment of tuberculosis in elephants.

There is a critical need for stakeholders to come together to focus on the sharing of information and to collaborate on detecting, diagnosing, treating, and ultimately reducing the impact of this devastating disease so that we may ensure a future for captive and wild elephants.

Keeping these aims in mind, a structured workshop approach was taken with a view towards developing specific goals, actions, responsible parties, and timelines in the broad areas of research, disease management, public relations and fundraising, and herd monitoring and herd management.

**PREVIOUS WORKSHOP SUMMARY**

There is historical and current evidence that elephants are susceptible to infection by MTB complex. Since 1996, the elephant display and veterinary communities have worked closely with the U.S. Department of Agriculture (USDA) to develop protocols for testing and treating elephants infected with MTB, as well as development of research priorities to learn more about potential risks and possible MTB transmission pathways (i.e. animal to animal, human to animal, and animal to human). There has also been an emphasis on putting the issue in context from both an animal and human health perspective.

There is a critical need for stakeholders to build relationships with and to integrate the efforts of USDA-APHIS Animal Care, USAHA, researchers, veterinarians, and animal managers to ensure the development of evidence-based best practices for managing TB in elephants. Through the two previous (Fort Worth 2011, Tulsa 2012) workshop formats, participants previously discussed currently available knowledge, focused on sharing information, and established potential areas of collaboration and common goals. They identified actionable items to improve detection, diagnosis, and treatment of MTB, with the objective of ultimately reducing the impact of this disease on the future of captive and wild elephants.

Key goals included 1) using science-based decision-making to develop practical, feasible and effective guidelines for the management and control of MTB in elephants; 2) defining MTB exposure; 3) determining the significance of a reactive ElephantTB STAT-PAK® and MAPIA test result in terms of risk of infection, and how definitions of these test results can best be reflected in the development of new guidelines that are practical, realistic, and effective in controlling MTB in elephants; 4) formulating public health recommendations 5) formulating recommendation for elephants that travel; and 6) developing short-term and long-range goals in the areas of research, disease management, herd monitoring, herd management, public health, funding, evidence-based policy and public relations.
3rd Annual Management and Research Priorities of Tuberculosis for Elephants in Human Care Workshop

FACILITATED BY JILL ALLREAD, PUBLIC COMMUNICATIONS INC. (PCI)

Jill Allread, APR, is president of Public Communications Inc. (PCI), a national communications firm in Chicago. She counsels a wide variety of clients to enhance their brand and reputation and to strengthen their internal and external communications by more effectively *telling their story* through strategic communications, planning, leadership coaching, and facilitation. A former newspaper reporter and editor, she joined PCI’s ownership team in 1994. Jill leads many clients through the process of strategic planning and she facilitates significant meetings and workshops for national and regional organizations and companies to help them build consensus and identify strategies for future success. She is a frequent spokesperson trainer and speaker on topics such as social media, board development and crisis management.

PROCESS

This facilitated workshop was designed to bring together the full range of stakeholders with a common interest in the conservation of the Asian and African elephant. Jill Allread used the process that promotes sharing of information and ideas. Structured analysis of problems was used to develop creative and inclusive solutions. Most of the workshop was spent working in small working groups, with occasional reports back to all participants in plenary sessions for comments and revision. This small group work allowed for effective and efficient use of time while plenary sessions allowed all participants to have input on all workshop recommendations.

This Workshop was designed to help participants achieve goals and actions to agreed upon objectives. In order to ensure that all participants had a common base of understanding, the workshop agenda included overview presentations from various workshop participants on research, disease management, and herd monitoring/herd management,

WORKSHOP GOALS

1) Information exchange  
2) Identify progress to date  
3) Challenges to address  
4) Priority/needs  
5) Updates for action plan  
   a. What is needed to advance efforts  
   b. How can we get it done
Sunday, August 25th

Session III Plenary – Topics identified by the group for further discussion.

Communication
  Increase stakeholder participation in all parts of the issue
  Improved collaboration
  Public information and finding one voice
  Define terms clearly
  Define terms to help communication and understanding, and ensure consistency
  Public health – information and communication about Mtb
  Public policy equals opportunities and challenges
  Build long term partners – for example human medical doctors
  Communication needs and risk
  Communication barriers with all group/levels
  Intra community communication
  Open lines of communication in group
  Trust
  International differences

Diagnostics and treatment
  Diagnostics not validated by science
  Discussion of diagnostic tests currently used
  What is the direction of infection – elephant to elephant, human to elephant, elephant to human
  Factors that indicate infection
  How to treat elephants for Mtb
  Disease management
  How test results affect ability to transport
  Better diagnostics
  Agree upon recommended protocols
  Misdiagnosis and responsibility to animals and public
  Serologic testing gives possible false positives
  Trunk wash has poor sensitivity
  DPP versus culture, how to react if positive
  Lab test results/resistance
  Ability to access diagnostic info
  Unsure if can be treated
  Treatment guidelines – may not be best for animals
  Cost of sourcing TB drugs
  Ability to access drug info
  Determine animal health status
  How to interpret past exposure
  Exposure versus disease defined
  What does exposure mean
  Correlation between trunk wash and Mtb
Treatment side affects
Impact of Mtb on wild elephant populations in range countries

Regulatory
- Regulator reaction to positive test (loss of control and how to treat)
- Other countries applying guidelines inappropriately
- Diagnosis results affect ability to transport and travel restrictions
- Following guidelines correctly or not per authority
- Regulatory ramification leads to misunderstanding
- Differing mandates/regulatory
- Guidelines don’t promote working together/miscommunication
- Issue used by animal activists against captivity
- Lack info to make decisions
- Unknown risk of moving animals
- Confusion between Mtb and M.bovis
- Regulatory results and effects
- Guidelines not validated science

Public Health
- Human health concerns – Occupational, Public
- Public information – finding one voices
- Perception by public
- Perception by regulatory community
- Consequence of public policy – trust and transparency
- Risks to staff, people, public
- Provide data regarding human risk and offer information to support message
- Communicate risk – define terms clearly

Session IV Working Groups – Attendees were randomly divided into working groups and allowed to discuss what they felt were the most important issues surrounding Mtb in elephants. Each group listed what they felt were the issues that gave them the most anxiety and needed the most advancement to help us understand and manage Mtb in elephants in the US, and then they listed their goals relating to these anxieties. These subjects were then openly discussed by the entire group in plenary.

Group 4

Anxieties
- Missing diagnosis of an infected animal and the personal responsibility to the animal, herd mates, collection, staff and public
- Regulatory ramifications
- Misunderstanding about public health and welfare
- Is treatment effective? Curative? we can never be sure?
- Regulatory and clinical mandates do not completely overlap
Different agencies have different mandates
  - State public health vet
  - State veterinarians
  - USDA Regulatory

Serologic testing is too heavily relied upon and many have seen serologic reactive, necropsy negative animals
Understanding of false positive, ‘infected latent’ and infected active, who needs treatment and who needs increased monitoring or possible prophylaxis
Trunk wash has poor sensitivity and no consistency how cultures are performed.
What we do in the U.S. and how it may be extrapolated or miscommunicated in other countries.
FOIA by animal rights’ groups has decreased cooperation and increased anxiety
Guidelines do not mitigate anxiety or help to balance the approach
Lack of confidentiality with our consultants and collaborators.

Goals
- Communicating what is known could allay a lot of these concerns.
- Both federal and state regulatory and clinical working together.
- Healthy elephants would relieve the anxiety between the traveling and stationary elephant holders so that we can work together.
- Cooperative projects from this group and increase trust so that collaborative research can progress
- Build trust such that cooperation across holders increases.
- Increase standardization of culture, and other methods across the industry.
- We either work together, zoo, circus, sanctuary and private or science will not move forward.
- Standardize communication to our own institution, keepers, curators, directors or lay people
- Develop position statements from the ECT and publicize them.
- Standardize treatment form, disseminate and share the form and the experiences with treatment.

Group 5

Anxieties
- Public perception to a positive animal
- Anxiety of regulator reaction to a positive test.
- Facilities that have elephants feel loss of control of what happens to or how they treat a positive elephant.
- Inappropriate generalization to our guidelines to other countries.
- How diagnostic test results affect facilities ability to transport animals.
- Following USDA guidelines correctly and ramifications of not doing so
- USDA interpretation of guidelines differ from facilities interpretation of guidelines, or state vet interpretation
Goals

- Communicate risk and define terms clearly to the public
  - Need to have or provide data about human disease risk that can help us inform the public and how we categorize the risk.
  - Develop information for general public about Mtb in elephants and treatment, website factsheet
- Educate officials and regulators about Mtb in elephants
- Access to USDA collection for analysis
- Publish studies on Mtb status on people who work with elephants to help inform occupational and public health risks.

Group 3

Anxieties

- Animal health, status of our animals,
- Use and interpretation of tests to determine their status and consequences of their test results and interpretation
- Treatment of the animals
- Issues of exposure, what does it mean or not mean.
- Human health, occupational but also public
- Perception by the public, what it all means or doesn’t mean, state, local, regulators, what does it mean to them or not.
- Real world consequences of the information we are generating regarding public policy, overall trust and transparency

Goals

- To be Mtb free in USA, and at least containment in developing countries.
- Optimal treatment and diagnostic management protocols
- Better identification of risks, actual, perceived and unknown
- Identification of consensus areas and communicate these consensus areas to outside world.
- Communication and transparency.

Group 2

Anxieties

- Makes people anxious due to emotional and charismatic species.
- Animal activists use it to promote elimination of captive elephants
- Working with partial knowledge or blanks so always second guessing ourselves
- Using this partial knowledge to control movement of animals - are we creating risk?
- Confusion of Mbovis vs Mtb,
• Being pushed technologically using things that are not validated or scientifically sound
• Treatment guidelines not being what is best for the animals.

Goals

• We need 4 pieces of epidemiology data: elephant to elephant, elephant to human, human to elephant and elephant to other animals transmission and infectivity information.
• Need clear communication, need website that is very clear, quick and easy.
• Need to look for alternative diagnostics,
• Need basic elephant research, immunology, physiology that will better help us understand Mtb in elephants.

Group 1

Anxieties

• DPP versus culture and how to react to the results if positive
• Anxiety over lab test results regarding resistance
• Perspective of the public over risk to animals, staff and public, transfer movement and financial risks to the institution
• Regulatory results that may affect transfer, travel restriction and financial risk to organization
• How to interpret past exposure amongst the elephant population
• Cost of sourcing Mtb drugs and diagnostic tests

Goals

• Antigen detection methods need to be developed.
• Systematic review of alternative diagnostics
• Standardize culture prep and methods for elephants
• Communication template to the public, via website, use the CDC’s precise wording to communicate to the public.
• Common message of open and direct communication template for institutions to use for the public.
• Increase the profile of this stakeholders group with regulators
• Facility design such that animals can be isolated, quarantined, etc
• Publicize guidelines to minimize exposure should a case come up.

Each attendee was asked to place their top two anxieties and concerns on a sticky note and submit them to the facilitator for organization.
Monday, August 26th

Session IV Working Groups – The previous day, workshop attendees worked collectively and in smaller breakout groups to identify the most urgent areas of concern that need to be addressed in order to make advances in elephant TB.

At the end of the first day, the facilitator summarized the sticky notes submitted by participants and organized them into five areas:

- Communications
- Diagnostics
- Guidelines
- Transmission
- Treatment

Communication needs and challenges were determined to be the highest priority for the group. Working groups made specific recommendations for ways to improve communication and to overcome barriers created by a breakdown in communications. Recommendations are as follows:

An important barrier to communication is trust and transparency. As a group, participants need to focus on science. We need to put the past in the past and recognize and celebrate the successes of this group.

**How to message**
- Message must be consistent
- Continued stream of information should be available
- Message should be proactive
- Should be a consensus among groups
- Should be communication between groups

**Who to communicate information to:**
Need to consider audience. Who is the audience we are attempting to reach?

- State veterinarians – We need to understand the concerns of state vets
  - a. Transporting of elephants
  - b. Potential risk exposure/transmission to livestock
  - c. Transport of elephants, especially movement of elephants that might be exposed but not monitored (tested)
  - d. Public health risk
- Regulatory agencies
- Government
- Elephant community
- General public
- Facility staff
- Administrators
- Colleagues
How to communicate information:
Organizations and agencies can disseminate the information
- USDA animal care
- AAZV
- USAHA
- AZVMA
- Elephant Care International
- International Elephant Foundation
- Elephant Managers Association
- AZA TAG
- NASPHV

Types of communication:
1) Layman fact sheets
   a) How do elephants get it
   b) Is the animal suffering
   c) Do wild animals get it
   d) What is being done
   e) Conservation implications
   f) How can I help
   g) Am I at risk
2) Technical fact sheets
   a) Defining terms
   b) Risks to people,
   c) Basic explanation of diagnosis
   d) Mtb and not M.bovis
   e) Testing – culture/serology and their strengths/weaknesses
   f) Treatment options and potential length of treatment
   g) How did elephant get infected? Suspect routes
   h) Paths of transmission to other animals
   i) Additional resources for those who want more info – resources/links, literature, reports,
   j) Acknowledge strengths and weakness of state of the science
   k) Sanitary protocols
   l) Transport
   m) How we manage the animals
   n) Different ways elephants are used (exhibit, public contact, rides, etc)
   o) Updating people resource list (additional people, correct info)
   p) Why treat – endangered species
   q) AAZV infectious disease fact sheets
   r) Establish how things are done like CLSI - Clinical and Laboratory Standards Institute
   s) Get buy-in with CDC /One Health
   t) OSHA like employee handout
Session VI Working Groups - Working groups reformed to identify issues and solutions to the remaining high priority items:

- Diagnostics
- Guidelines
- Transmission
- Treatment

It was noted that in order to identify issues and solutions, we need to be communicating effectively and that means that we must define terms.

**Defining terms**
1. Subcommittee should be formed to generate and solicit feedback on working definitions
2. Use CDC definition of exposure, active infection and Mtb exposure.
3. Use Elephant Care International - website already well done

**Terms**
1) Active disease
2) Contagious
3) Epidemiological terms (eg prevalence, incidence)
4) Exposure –
   - not directly related to infectious /contagious
   - sharing common airspace with a known culture positive
   - any situation in which an individual is in direct or indirect (shared space, food or water source or housed adjacent to an elephant enclosure) with M.tb complex organisms, or an M.tb infected animal (e.g. M.tb infected elephant, human, or other animal)
5) Infection – discuss spectrum of infection
6) Inactive infection
7) Infected/diseased
   - bacteriologic evidence of Mtb through culture, genexpert
   - an elephant from which mycobacterium tuberculosis complex has been identified through culture, PCR and other molecular techniques
8) Latency, Latent infection
9) M.tb complex – m. tuberculosis, mbovis, m. africanum, m.microti, m.caneth, m.capra, m.pinnipedii
10) Mtb - Infection with a Mycobacteria species of Mtb complex
    a. Cattle can get Mtb dead end host, it’s shown only to be transmitted by close contact
    b. Need to get that information for cattle Mtb infection
11) Seropositive
12) Ante Mortem Definitions:
    - Active Infected Elephant: An elephant that has been identified as having tuberculosis via positive microbial culture, PCR or other antigen based molecular techniques from an ante mortem sample.
    - Latent Infected Elephant: An elephant that has no shedding of the organism, showing no signs of clinical disease but has a confined or walled off granuloma.
• Immunologically Reactive Elephant: An elephant that has detectable humoral antibodies against Mtb antigens. But we felt stat-pak reactive alone without MAPIA or DPP was not a reactive positive.

13) Post Mortem Definitions:
• Actively or Infectious Elephant: An elephant that upon post mortem exam has active disease in an organ system with communication to the outside.
• Latently infected Elephant: An Elephant that upon post mortem exam has a confined or walled off granuloma with no communication to the outside.
• Need a standardized review of necropsy material, including histopathology and histochemistry. Definition of lesions.
• Exposed Elephant: An elephant that has had direct contact to an animal that has been shown to be shedding at the time of their contact at any time in its lifetime.

Diagnostics
1) Predictive value of current ante-mortem tests is not high enough across general population. Careful attention to reliability and testing factors, host factors that affect test performance associated with trunk wash.
2) Lack of available serology tests also affects our ante-mortem testing efforts.
3) Stat-pak results should not be reported independently, the Stat-pak is only a screening tool and should trigger a MAPIA or DPP.
4) Stat-pak results should not change the definition of an elephant but should make clinician consider increased surveillance of elephants with an antigen/agent based test.
5) Currently available ante mortem diagnostics, both trunk wash and serology have incompletely defined performance characteristics therefore it is imperative to develop new, adjunct and multi-pronged approach to diagnosis of elephant Mtb
6) Basic explanation of diagnosis
7) Treatment options and potential length of treatment
8) Transmission to other animals
9) How did elephant get infected? Suspect routes
10) Immunologically reactive – MAPIA positive or STATPAK positive or DPP positive
11) Infection is not same as contagious
12) Infection is a spectrum
13) What are these “screening tests” doing
14) What information are these tests giving
15) What does a positive culture mean
16) Terms need to be defined
17) Need to be as evidenced-based as possible
18) Protocols
19) Reliable tests
20) Collaborative efforts
21) DPP versus culture
22) Antigen versus antibodies
23) Need access to all data
24) Direct lavage – Tom H
25) “Sweet string” – Jennifer
26) PCR – New genexpert, National Jewish hospital
Transmission
1) Does elephant actually aerolize
   • need to determine particle size for transfer to elephant,, human, other animals
2) Transmission /epidemiology
   • Factors
   • Elephant to elephant
   • Occupational risk
3) No documented cases of elephants to livestock
4) No documented cases of elephant to general public
5) Evidence/concern of an occupational health risk
6) In general Mtb strains found in elephants are strain commonly found in humans
7) Current observation /info would suggest that Elephant to Elephant transmission is not rapid, does not spread rapidly among captive elephant populations
8) Based on current info the majority of Mtb culture positive captive elephants do not manifest clinical signs

Treatment
1) Treatment versus successful treatment
2) No idea what we are treating, no idea when we are done, when we are successful, what stage disease is
3) What is successful treatment
   • Treatment completed – x doses in x days
   • Treatment not completed because
     ➢ Adverse events/intolerance
     ➢ Refuse to take /cannot ingest
     ➢ Death
   • Treatment in progress – x doses x days
4) How many drugs, how long, how much, how to monitor
   • Elephant on treatment can be moved if blood MK on 1 sample – this is true for humans (nardell et al 2012 AJRCCM)
   • FQ can be substituted for INH or RIF with + equivalence – (Johnson et al 2008 IJRD)
5) Research when shedding stops
6) Test that measures treatment response
7) Develop clinical hand book for practical advice for providers
   “So your elephant has TB…”

Guidelines
1) Guidelines are developed/continuing to be updated but slow to accept and implement so it is confusing as to which guideline to use
2) Name of USAHA guidelines by year is confusing so propose change name of guidelines to version 1,2,3
3) Need input based on science and stakeholders
4) Stakeholders should produce the guidelines as it is done for other stakeholder groups within the USAHA committees
5) Define and describe the USAHA process for these guidelines
6) Encourage the Elephant TB subcommittee to re-evaluate movement advice based on serological test results
7) Take out serologic positive trunk wash negative travel restrictions
8) Recommended to review the entire document
9) USAHA developed guidelines for infection is only a culture positive animal.
   2010 guidelines state an infected is Statpak/MAPIA reactive
   2012 – 2008 guidelines state an infected animal is only culture positive.

CONCLUSIONS

This facilitated workshop brought together a range of stakeholders with a common interest in the conservation of the Asian and African elephant. Most of the workshop was spent working in small working groups, with reports back to all participants in plenary sessions for comments and revision. The group work allowed for effective and efficient use of time identifying concerns, needs and future actions while plenary sessions allowed all participants to have input on each issue. All issues in this document were presented to all participants, and each participant had multiple opportunities to comment on issues and each response was noted.

From these discussions and notes, workshop actions were identified and a process initiated to achieve outcomes that will advance knowledge and treatment of Mtb in elephants, and to communicate this knowledge to peers, government officials and the general public.

Workshop actions identified
- Define terms used to talk about elephant TB and have the definitions be scientific based
- Proactively poll state veterinarians & public health officials for their input regarding concerns about elephant TB and what kind of information they feel they need
- Research and collect existing data, studies and information and make it available
- Develop fact sheets to ensure consistent messages and to have the information available to help inform stakeholders, officials and the public. Topics for fact sheets include:
  - Basic information about TB
  - A list of frequently asked questions and answers
  - A fact sheet for elephant keepers/handlers, managers
  - A fact sheet for policymakers on the issues
  - A media fact sheet
- Identify experts and including them in a resource list that is available
- Create a website or webpage that provide information that can also be linked to other existing resources
- Develop consistent messages and share them with stakeholders
- Create a mission or purpose statement for the Elephant Care Taskforce
- Provide quarterly or semi-annual updates of information to help bridge information gaps between workshops
- Develop a diagnostic tool for antigens
- Systematic review of diagnostic
- Develop better communication and increased credibility with regulators
Define and describe the USAHA process for informative, transparent and inclusive input into the development of animal health recommendations, including the Elephant TB guidelines document

Process for addressing needs and term definitions
- Establish a task force or committee that:
  - represents multi-disciplines
  - is science based
- Begin by referencing guidelines to do comparisons of definitions currently in use
- Goal: write a science paper that can be published and provides consensus definitions
- Begin today to address barriers, advance progress through improved communication

Terms the group suggested defining to help ensure consistent communication and understanding are:
- Exposure
- Active case
- Successful treatment
- Contact with an animal known to be positive
- Latency
- Positive culture
- Infection vs. contagious
- Prevalence
- Incidence
- Truck wash
- Transmission

Assignments
1. Elephant epidemiology survey in US - in progress
2. Occupations risk assessment – analysis and publication in progress
3. Elephant epidemiology survey in Kerala, India – in progress
4. Occupational health survey in Kerala, India – in progress
5. Droplet size study in Kerala, India and US - planned
6. Statement about use of serologic tests and USA guidelines in other countries.
7. Develop position statements, FAQs for websites and listservs
8. Develop stake holder’s resource for sample requests for collaborative efforts. This will be maintained by steering committee designee or list-serve for sample requests.
9. Individuals in the group have identified scenarios to validate Genexpert retrospective tissue and TW culture (+) and (-) tissues.
10. Need Vivionne to participate with known (+) samples.
11. PCR results should be confirmed with sequenced
12. Crisis Management - Portland zoo did a good job of crisis management
13. Consensus definition publication
14. Publish Mtb genotypes found in captive elephant population in US
15. Systematic literature review
16. Encourage the Elephant TB subcommittee to re-evaluate movement advice based on serological test results
17. Contact Dr. Adam Langer of CDC (ak17@cdc.gov). Name provided by Ken Castro, head of the division of TB elimination to Jennifer Furin.

**Michele and Ramiro** - Define scientific based terminology as part of epidemiological survey

**Kay**
- Ask Bob and Dustin process for polling state vets and public health officials
- Develop 3 concerns of state vets written and responses
- Report to AAZV executive Committee and ask for funding for next year.

**Julia Murphey** - with contact to NASPHV and CDC vet our public health information

**Deborah Olson** – Develop Mission and Purpose statement for ECT.
1) Human side
2) Animal side
3) Presentation side

**Chris Stemme** - Develop range country statements about whole health, position on wanting to prevent the spread of elephant TB to wild populations through good whole health preventative medicine, and statement that use of serology out of the context of a whole health preventative health strategy is unethical.

**AJ Marlar and Jennifer Furin** - research protocol draft abstract to steering committee ~ 6 months.

**Jennifer Fuin** –
- Develop connection to human experts
- Health and you at the zoo

**Thomas Hildebrandt** - short abstract update to ECT steering committee on use of lung lavage in elephants at next year’s TB workshop in St. Louis.

**Michele Miller** - Inquire to Scott Terrell if he would be willing to review and standardize elephant tissue cases.
Tuberculosis Facts (prepared by Jennifer Furin after the workshop)

- Tuberculosis (TB) is a disease of the lungs (usually) that is caused by a bacteria. It is primarily a human disease and it is spread through the air.
- Most people who are infected with the bacteria do NOT get sick unless they have a problem with their immune systems. Globally, 2.75 billion people are infected with TB and most have a only a ten percent lifetime risk of developing TB disease.
- Tuberculosis is a treatable disease. With six months of treatment using four drugs, more than 95% of people will be cured and go on to lead normal, healthy lives.
- Factors that affect TB exposure and infection are the length of exposure, the intensity of the exposure, and the setting in which exposure occurs. In general, people who are exposed over long periods of time (> 8 hours) in close quarters (i.e. sharing living space) in a setting with low air circulation (i.e. indoors, no windows, no air circulation).
- An airplane, for example, is a setting in which TB transmission might occur because of its closed quarters and lack of air circulation. The World Health Organization has issued a set of guidelines on TB transmission in airplane settings and only recommends screening people who have been exposed to a person with TB deemed to be infectious, who have been on flights for longer than 8 hours, and who sat either in the same row or two rows in front and behind. It is also recommended that flight crew be screened.
- Elephants can sometimes become infected and sick with TB, usually because a human has passed it along to the elephant.
- While theoretically elephants could pass TB to the general public through the air, the degree of infectiousness of the elephant, length of exposure (usually less than 8 hours for the general public) and setting of exposure (usually outside) make transmission unlikely to occur.
- There have been no cases of TB in the general public that have been linked to exposure to a sick elephant.
- For these reasons, public health officials and doctors do NOT consider the general public to be at risk, even after exposure to an elephant with TB.
- More common health concerns for people visiting zoos, circuses, and other elephant viewing areas are the spread of human diseases, including flu, the common cold, and certain viruses that cause diarrhea. Frequent hand washing is recommended to prevent these infections in the general public.
- For more information on tuberculosis, please refer to the following websites:
  - [http://www.cdc.gov/TB/](http://www.cdc.gov/TB/)
  - [http://www.who.int/topics/tuberculosis/en/](http://www.who.int/topics/tuberculosis/en/)


viii http://www.oregonlive.com/health/index.ssf/2012/12/about_70_sickened_at_oregon_zo.html
SOME CONSIDERATIONS ON MYCOBACTRIUM TUBERCULOSIS IN ELEPHANTS IN ASIAN RANGE COUNTRIES

Christopher Stremme
Veterinary Society for Sumatran Wildlife Conservation
SUMATRA / INDONESIA
SUMATRA / INDONESIA
POST MORTEM
SUMATRA / INDONESIA
POST MORTEMS
SUMATRA / INDONESIA
Lab Results

3 different tissue samples from the lung were used for culture and Ziel-Neelsen acid fast stain -> all three samples negative

A different lab conducted PCR and Ziehl – Neelsen from lung, kidney and liver tissue samples -> both positive

PCR positive for *M. tuberculosis* and *M. bovis*

<table>
<thead>
<tr>
<th>Kolom</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>(marker 100 bp)</td>
</tr>
<tr>
<td>kolom 1</td>
<td>(Kidney)</td>
</tr>
<tr>
<td>kolom 2</td>
<td>(Lung)</td>
</tr>
<tr>
<td>kolom 3</td>
<td>(Liver)</td>
</tr>
<tr>
<td>kolom 4</td>
<td>(<em>M. tuberculosis</em>)</td>
</tr>
<tr>
<td>kolom 5</td>
<td>(<em>M. bovis</em>)</td>
</tr>
<tr>
<td>kolom 6</td>
<td>(nuclease free water)</td>
</tr>
</tbody>
</table>
## EPIDEMIOLOGY

### ESTIMATED WHO REGIONAL TB STATISTICS FOR 2011

<table>
<thead>
<tr>
<th>Region</th>
<th>Incidence</th>
<th>Prevalence</th>
<th>Deaths</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>2,300,000</td>
<td>2,500,000</td>
<td>220,000</td>
<td>857,382,000</td>
</tr>
<tr>
<td>Americas</td>
<td>260,000</td>
<td>330,000</td>
<td>21,000</td>
<td>943,019,000</td>
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<tr>
<td>Eastern Mediterranean</td>
<td>660,000</td>
<td>1,000,000</td>
<td>99,000</td>
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<tr>
<td>Europe</td>
<td>380,000</td>
<td>500,000</td>
<td>45,000</td>
<td>899,500,000</td>
</tr>
<tr>
<td>South-East Asia</td>
<td>3,500,000</td>
<td>5,000,000</td>
<td>480,000</td>
<td>1,830,361,000</td>
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<tr>
<td>Western Pacific</td>
<td>1,700,000</td>
<td>2,500,000</td>
<td>130,000</td>
<td>1,808,797,000</td>
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<tr>
<td><strong>Global Total</strong></td>
<td><strong>8,800,000</strong></td>
<td><strong>11,830,000</strong></td>
<td><strong>995,000</strong></td>
<td><strong>6,947,687,000</strong></td>
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</tbody>
</table>

The figures for the number of deaths exclude the deaths of people who had both TB and HIV infection at the time of their death.
<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>INCIDENCE</th>
<th>PREVALENCE</th>
<th>DEATHS</th>
<th>POPULATION</th>
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<tbody>
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<td>Afghanistan</td>
<td>61,000</td>
<td>110,000</td>
<td>13,000</td>
<td>32,358,000</td>
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<td>620,000</td>
<td>68,000</td>
<td>150,494,000</td>
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<tr>
<td>Brazil</td>
<td>83,000</td>
<td>91,000</td>
<td>5,600</td>
<td>196,655,000</td>
</tr>
<tr>
<td>Cambodia</td>
<td>61,000</td>
<td>120,000</td>
<td>9,100</td>
<td>14,305,000</td>
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<tr>
<td>China</td>
<td>1,000,000</td>
<td>1,400,000</td>
<td>47,000</td>
<td>1,347,565,000</td>
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<tr>
<td>DR Congo</td>
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<td>350,000</td>
<td>36,000</td>
<td>67,758,000</td>
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<td>200,000</td>
<td>15,000</td>
<td>84,734,000</td>
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<tr>
<td>India</td>
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<td>300,000</td>
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<td>680,000</td>
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<td>242,326</td>
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<td>Kenya</td>
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<td>120,000</td>
<td>9,200</td>
<td>41,610</td>
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<td>Mozambique</td>
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<td>120,000</td>
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<td>240,000</td>
<td>23,000</td>
<td>48,337,000</td>
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<td>190,000</td>
<td>280,000</td>
<td>27,000</td>
<td>162,471,000</td>
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<tr>
<td>Pakistan</td>
<td>410,000</td>
<td>620,000</td>
<td>59,000</td>
<td>176,745,000</td>
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<tr>
<td>Philippines</td>
<td>260,000</td>
<td>460,000</td>
<td>28,000</td>
<td>94,852,000</td>
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<tr>
<td>Russian</td>
<td>140,000</td>
<td>180,000</td>
<td>22,000</td>
<td>142,836,000</td>
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<tr>
<td>Federation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>500,000</td>
<td>390,000</td>
<td>25,000</td>
<td>50,460,000</td>
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<tr>
<td>Thailand</td>
<td>86,000</td>
<td>110,000</td>
<td>9,800</td>
<td>69,519,000</td>
</tr>
<tr>
<td>Uganda</td>
<td>67,000</td>
<td>63,000</td>
<td>5,000</td>
<td>34,509,000</td>
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<tr>
<td>UR Tanzania</td>
<td>78,000</td>
<td>82,000</td>
<td>6,400</td>
<td>46,218,000</td>
</tr>
<tr>
<td>Viet Nam</td>
<td>180,000</td>
<td>290,000</td>
<td>30,000</td>
<td>88,792,000</td>
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<tr>
<td>Zimbabwe</td>
<td>77,000</td>
<td>70,000</td>
<td>6,000</td>
<td>12,574,000</td>
</tr>
<tr>
<td><strong>Total for High Burden Countries</strong></td>
<td><strong>7,053,000</strong></td>
<td><strong>9,696,000</strong></td>
<td><strong>821,000</strong></td>
<td><strong>4,370,720,000</strong></td>
</tr>
</tbody>
</table>
Number of MDR-TB* cases estimated to occur among notified pulmonary tuberculosis cases, 2011

* MDR-TB: multidrug-resistant tuberculosis (resistance to, at least, isoniazid and rifampicin)

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

**DIAGNOSTICS**

**SEROLOGY**: Stat-Pak, MAPIA, DPP
Lack in sensitivity and specificity
Even accurately positive serology test does not allow to conclude an active TB infection only tells that a person has been affected with TB bacteria.
It does not tell whether the person has *latent TB* (LTBI) or has progressed to *active TB diseases*.
People with latent TB infection are not infectious and cannot spread TB bacteria to others.

**CULTURE**: trunk wash
Lack in sensitivity,
Need of well-trained animals and handlers
Limitations in storage and shipment of samples,
Limitation in appropriate lab facilities in many rural regions in Asian range countries
INDIA

- Early Hindu literature furnishes the evidence that TB was encountered.
- Sporadic reports about TB in captive elephants since 1910.
- In publications up to 2011 the use of intradermal tuberculin test for TB diagnosis is still suggested for Elephants.
INDIA

The important Causes of mortality in a study in wild elephants in Kerala from 2007 – 2001,

- Predation
- Poaching
- Human Elephant Conflict
- Natural reasons
- Diseases
  - Out of 88 postmortem 2 case positive for TB
  - Impression smear, Culture, histopathology and PCR
  - First case of TB confirmed in wild elephants
  - Spill over from humans suspected as source of the infection (the *M.tb* strain belonged to East-African-Indian predominant strain of human TB in Southern India)
INDIA

South India (Kerala, Karnataka, Tamil Nadu)
Several TB cases from post mortem reported by different authors.
Many controversial discussion amongst different regional elephant veterinarians about diagnostic, prevention and treatment of TB in the captive population.
High incidence amongst the human population and close interaction between humans and captive elephant seen as major source of TB infections in the captive elephants
ASSAM: A study in Assam report about 2 TB cases out of 88 individual in captive elephants.

“Tuberculosis is a major problem in undernourished elephants living in unhygienic and overcrowded places.”

“The mahouts should be tested annually as they may be the main carries.”

“In Assam elephants, TB has not been a major problem so far, our biggest problem (in Assam) is still parasites”

*Kushal K. Sarma 2011*
Thailand

A serology survey using TB Stat-Pak was conducted using samples from 803 captive elephants collected between 2005 and 2008. Stat-Pak tests from 52 elephant were reactive, but overall the study demonstrated a very poor correlation between results gained from the Stat–Pak test and ELISA results from the same sample set.
Thailand

In a study on 4 elephant showing clinical signs suspicious for TB ante mortem tests included multiple STAT-PAK and culture, 3 of these elephants were positive for mycobacteria in tissue culture at necropsy, showed that bacterial cultures (one elephant still alive). The ante mortem STAT-PAK conducted at different times showed reactive and non-reactive results in 2 of these and were always non-reactive in one of these animals. Out of 60 ante mortem culture from trunk washes, 2 were positive for mycobacteria.

“The study indicates that serologic tests or other diagnostic procedures could not unequivocally identify infected animals...”

“On the basis of these molecular studies, we believe that *M. tuberculosis* was probably transmitted to these 4 elephants from humans.”

Taweepeoke Angkawanish et al 2010
A senior vet from the MTE says TB in the about 2,800 MTE elephants, over a period of time of 10 years, has been reported to have been 2 cases where clinical symptoms and post mortem finding were suspicious for TB. Therefore he feels that TB is not a significant problem in MTE elephants. He reports some mahouts had tested positive for TB during health check in local hospitals.

Another former MTE veterinary officer reports that confirmed deaths from TB have occurred in working elephants. Means of diagnostics are not reported, prevalence and incidence unknown.
In a Study 87 serum samples of 40 free ranging adult and sub adult elephants and from 47 orphaned juveniles were assayed using TB STAT-PAK. Samples from 2 of the free ranging elephant and 15 from the orphaned were reactive. No other Serological (DPP, Mapia, ELISA), culture or PCR tests were conducted for validation of these results.

In 2002 TB was diagnosed the first time by culture from trunk wash sample and confirmed by post mortem findings. In principle the trunk wash sample is considered not very practical for captive elephants due to lack of specific training for this procedure.
LAOS

A study in captive elephants using TB STAT-PAK reported to have reactive results >20%.

No other Serological (DPP, Mapia, ELISA), culture or PCR tests were conducted for validation of these results.

Very low incidence of TB amongst mahouts in this study.
NEPAL

TB was first identified in Nepal in 2002.

Between 2002 and 2009 deaths in which TB was diagnosed.

Since 2006 starting a TB surveillance program based on different serology tested mainly Stat-Pak and DPP.

Based on the results of this surveillance method about 23% of the captive population of around 200 animals are judged as infected with TB.

In 2008 over 100 elephant handlers were tested for TB, but no cases of TB were detected.
NEPAL
First government program for the control of a specific infectious disease in elephants

BUT

1. The entire diagnostic decisions about prevention and treatment schemes, separation and quarantine conditions is based only on 2 serology tests (Stat-Pak and DPP) with proven to lack in its specificity and sensitivity. No appropriate evaluation for the differentiation between latent infections and active TB is made.

2. Only single disease approach, no holistic approach to the entire elephant health care and management needs and thus putting the attention only on one single disease in captive elephant health
Importance of TB in relation to other diseases in Asian elephants

Questionnaire amongst 45 veterinarians from 8 range countries

<table>
<thead>
<tr>
<th>SYNDROMES-MORBIDITY</th>
<th>SUM OF SCORE</th>
<th>SCORE %</th>
<th>SYNDROMES-MORTALITY</th>
<th>SUM OF SCORE</th>
<th>SCORE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury</td>
<td>167</td>
<td>27</td>
<td>Injury</td>
<td>192</td>
<td>35</td>
</tr>
<tr>
<td>(Gunshot wounds)</td>
<td>(11)</td>
<td>(2)</td>
<td>(Gunshot)</td>
<td>(12)</td>
<td>(2)</td>
</tr>
<tr>
<td>Parasitism</td>
<td>100</td>
<td>16</td>
<td>Infectious disease, not due to parasitism</td>
<td>83</td>
<td>14</td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>78</td>
<td>13</td>
<td>Gastrointestinal disease</td>
<td>29</td>
<td>9</td>
</tr>
<tr>
<td>(Diarrhea)</td>
<td>(27)</td>
<td>(4)</td>
<td>(Diarrhea)</td>
<td>(23)</td>
<td>(4)</td>
</tr>
<tr>
<td>Injury</td>
<td>167</td>
<td>27</td>
<td>Injury</td>
<td>192</td>
<td>35</td>
</tr>
<tr>
<td>(Gunshot wounds)</td>
<td>(11)</td>
<td>(2)</td>
<td>(Gunshot)</td>
<td>(12)</td>
<td>(2)</td>
</tr>
<tr>
<td>Parasitism</td>
<td>100</td>
<td>16</td>
<td>Infectious disease, not due to parasitism</td>
<td>83</td>
<td>14</td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>78</td>
<td>13</td>
<td>Gastrointestinal disease</td>
<td>29</td>
<td>9</td>
</tr>
<tr>
<td>(Diarrhea)</td>
<td>(27)</td>
<td>(4)</td>
<td>(Diarrhea)</td>
<td>(23)</td>
<td>(4)</td>
</tr>
<tr>
<td>Ocular disease</td>
<td>55</td>
<td>9</td>
<td>Poisoning</td>
<td>46</td>
<td>8</td>
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<tr>
<td>Foot pathology</td>
<td>48</td>
<td>8</td>
<td>Old-age related</td>
<td>44</td>
<td>7</td>
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<tr>
<td>Malnutrition</td>
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<td>7</td>
<td>Nonspecific</td>
<td>39</td>
<td>7</td>
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<tr>
<td>Abscess</td>
<td>33</td>
<td>5</td>
<td>Parasitism</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>Infectious disease, not due to parasitism</td>
<td>33</td>
<td>5</td>
<td>Malnutrition</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Lameness</td>
<td>19</td>
<td>3</td>
<td>Renal disease</td>
<td>10</td>
<td>2</td>
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<tr>
<td>Skin disease</td>
<td>7</td>
<td>1</td>
<td>Cardiac disease</td>
<td>6</td>
<td>1</td>
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<tr>
<td>Stereotypical behavior</td>
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<td>1</td>
<td>Hemorrhagic disease</td>
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<td>Over work</td>
<td>4</td>
<td>1</td>
<td>lack of veterinary care</td>
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<td>1</td>
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<tr>
<td>Poisoning</td>
<td>4</td>
<td>1</td>
<td>Lameness</td>
<td>5</td>
<td>1</td>
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<tr>
<td>Renal disease</td>
<td>4</td>
<td>1</td>
<td>Musth</td>
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<td>Prolonged recumbency</td>
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<tr>
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<td>Seizure</td>
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<td>Reproductive problem</td>
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<td>1</td>
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<tr>
<td>Chemical immobilization</td>
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<td>Total</td>
<td>100</td>
<td></td>
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## Importance of TB in relation to other diseases in Asian elephant

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<thead>
<tr>
<th>REPORTED POST-MORTEM PATHOLOGIC FINDINGS</th>
<th>SUM OF SCORES</th>
<th>%</th>
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<tr>
<td>Human elephant conflict (electrocution, poisoning, poaching, train collision, gunshot, wells, pit traps, snares, etc.)</td>
<td>53</td>
<td>10.4</td>
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<tr>
<td>Hemorrhage</td>
<td>52</td>
<td>10.2</td>
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<tr>
<td>Endoparasites</td>
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<td>7.5</td>
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<tr>
<td>Gastrointestinal stasis or torsion</td>
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<td>6.7</td>
</tr>
<tr>
<td>Lung lesions</td>
<td>33</td>
<td>6.5</td>
</tr>
<tr>
<td>Liver lesions</td>
<td>22</td>
<td>4.3</td>
</tr>
<tr>
<td>Old age</td>
<td>21</td>
<td>4.1</td>
</tr>
<tr>
<td>Undetermined</td>
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<td>4.1</td>
</tr>
<tr>
<td>Injuries from intraspecific aggression</td>
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<td>3.9</td>
</tr>
<tr>
<td>Toxin</td>
<td>18</td>
<td>3.5</td>
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<tr>
<td>Splenic lesions</td>
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<td>3.5</td>
</tr>
<tr>
<td>Tetanus</td>
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<td>3.5</td>
</tr>
<tr>
<td>Cardiac lesions</td>
<td>17</td>
<td>3.3</td>
</tr>
<tr>
<td>Renal lesions</td>
<td>17</td>
<td>3.3</td>
</tr>
<tr>
<td>Enteritis</td>
<td>16</td>
<td>3.1</td>
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<tr>
<td>Traumatic injuries</td>
<td>16</td>
<td>3.1</td>
</tr>
<tr>
<td>Sepsis</td>
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<td>2.7</td>
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<tr>
<td>Emaciation</td>
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<td>2.4</td>
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<tr>
<td>Skin lesions</td>
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<td>2.2</td>
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<td>Rabies</td>
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<td>1.4</td>
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<tr>
<td>Tuberculosis</td>
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<td>1.4</td>
</tr>
<tr>
<td>Elephant entotheliotropic herpes virus</td>
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<td>1.4</td>
</tr>
<tr>
<td>Autolysis</td>
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<td>1.2</td>
</tr>
<tr>
<td>Cyanosis</td>
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<tr>
<td>Peritonitis</td>
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<td>1.0</td>
</tr>
<tr>
<td>Lightning</td>
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<tr>
<td>Arthritis</td>
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<td>0.8</td>
</tr>
<tr>
<td>Anemia</td>
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<td>0.6</td>
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<tr>
<td>Nasal and oral mucosa ulceration</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>Abscess</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>Eye conditions</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>GRAND TOTAL</strong></td>
<td><strong>510</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
What is the situation for TB in Elephants in Asia

- Single cases have been reported and confirmed from captive elephants and several from wild elephants in one range country (mostly from post mortems).
- True prevalence and incidence is unknown, all studies involving larger number of animals using serology, mostly only STAT-PAK.
- Epidemiological details are unclear.
- High human incidence and prevalence in the range countries makes it likely for humans to be the major source of infection.
- Horizontal infection amongst elephants and transmission from elephant to humans not confirmed yet.
- The presence of TB in elephants does not seem to play a role for TB Epidemiology in the human population.
What is the situation for TB in Elephants in Asia

- No diagnostic methods for live animals with reliable sensitivity and specificity available.
- Limited lab capacities for culture, PCR and histopathology
- Limited level of knowledge about different diagnostic methods, its limitations and interpretation of results amongst veterinary staff.
- **TB is not the most important health issue in Asian Elephant in the range countries**
- **TB is not the most frequent cause of mortalities in Asian Elephants in range countries**
- Lack of attention to basic health management needs, (malnourishment, chronic wound and abscess, high parasite infestation, poor hygiene, overwork) very often causes poor general health condition, making animals susceptible for different kinds of infectious diseases
WHAT DO WE NEED TO IMPROVE THE HEALTH OF CAPTIVE ELEPHANTS AND PREVENT INFECTIOUS DISEASES (incl. TB) IN ASIAN RANGE COUNTRIES

A holistic approach for a preventive health management, approaching the major and most basic health issues to achieve a very high average health condition of the elephants in order to reduce their susceptibility for any kind of infectious diseases.

Health surveillance, welfare and education programs for elephant handlers and their families

Reducing the direct interaction between the general public and elephants

Develop better diagnostic methods and treatment for TB and other infectious diseases
THANK YOU FOR YOUR ATTENTION
TB MANAGEMENT
WHAT SHOULD NOT HAPPEN

DR FLORENCE OLLIVET-COURTOIS, DVM, ZOO AND WILDLIFE CONSULTANT
NEPAL, BABY AND JAVA

- All 3 animals were imported young from Asia
- worked during many years in different circus
- Java belongs to the zoo, Baby and Nepal to a circus
- lived in different enclosures separated by a moat
• 1999: arrival at Lyon zoo after a fight with 2 other elephants. No training problem observed.
• 2005 anesthesia of Java for hoof trimming. Blood samples done and stored, all disappeared.
• 2009 anesthesia of Népal for X-rays of the elbow. Blood sample done and stored, samples of the zoo disappeared.
• The zoo managed the elephants without training and, without contact and decided to start with protected contact in 2010
• Fifteen keepers take care of the elephants
• It takes 6 months to take the first blood samples
FIRST PROBLEMS IN 2010

- July and August MAPIA and ERT tests done on Baby and Nepal, not in Java
- No trunk wash done
- Sanitary authorities warned but takes no measure
- October: the zoo asks Baby and Nepal to be euthanised
- January 2011, Sanitary authorities ask animals to be isolated but no new tests, and advise euthanasia
## SUMMARY OF TESTS

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>ERT</td>
<td>reactive</td>
<td>reactive</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>ELISA</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>MAPIA</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>DPP expérimental</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>reactive</td>
<td>negative</td>
<td>reactive</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>experiment</td>
<td>not validated in 2010</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Group 3 A: Culture negative; STAT-PAK® reactive; MAPIA™/DPP® nonreactive; no known exposure

Culture q 3 months 1st year; q 6 months next 2 years then annually if cultures negative and MAPIA™/DPP® remains nonreactive; repeat MAPIA™/DPP® q 6 months for 1st year
No treatment or travel restrictions
• The zoo refuses to test java
• Zoo stops training for blood samples
• Zoo refuses to train for trunk wash
• The owner refuses euthanasia and asks for new exams
AND M.TUBERCULOSIS IS DIAGNOSED

• August 2012 Java dies
• 10th of December 2012: culture positive M. tuberculosis
• 11th of December euthanasia ordered
• The court of appeal confirms euthanasia
• Brigitte Bardot and Princess Stephanie of Monaco ask the French président to help
• The zoo refused to do new tests because the animals were dangerous,
• MAPIA negative results of 2010 not transmitted to the court and experts
• Animals administratively contaminated
• In France euthanasia has always been the rule for TB suspicion in animals
• False negative tests « more frequent than false positive »
• Animals not trainable
• Animals have no conservation value
POLITICIAN INVOLVED

• French president and Minister of Agriculture decided to stop euthanasia and wait for the last court of appeal decision
• The local authorities refuse and maintain the decision of euthanasia
• February 2013 the last court of appeal decides euthanasia is not legal:
  • No independant evaluation
  • The law offers other possibilities to deal with such situation (new tests and isolation)
• ANSES : « Animals should be tested » (no time limit !)
• A new sanitary facility built by princess of Monaco
• Treatment forbidden
If tests are negative they are false negative results.
If tests are positive, these are true positive.
Real sensitivity and specificity are not known.
So why ANSES is asking new tests?
Page 18

“the fate of Lyon zoo elephants, old and maybe sick, have no interest

but capturing animals from the wild for circus or zoos will worsen the delicate situation of this species”!
TESTS DONE IN JULY 2013

- Elephants moved in July 2013 to south of France near Monaco
- DPP tests negative in both elephants
- PCR and CUTURE pending
Nepal Elephant Healthcare and TB Surveillance Program

Hank Hammatt, Susan Mikota DVM
Elephant Care International
How do we measure... the worth of protecting wild herds or of an individual elephant?

A recent article in Gajah* said that “large sums of funding” ...and “large amounts of resources” are spent on the diagnosis, treatment, and management of TB, diverting potential resources from conservation.

* Riddle, Miller, Schmitt 2012
The Worth of an Elephant?

- New baby ele to a zoo ~ $1,000,000
- Houston Zoo paid $500,000 for a 27-year-old female & a 4-year-old male elephant
- U.S. Zoos pay ~ $50,000 for TB drugs / ele
- Value of a trained patrol elephant in Nepal protecting elephants, rhino...?
Large sums of funding on TB

- $40 million for zoo facility for 6 elephants
- Vs < $400,000 on a multi-year Nepal TB Program affecting > 150 elephants
- Considering the value of a single patrol elephant, we, and the Government of Nepal, recognize the Plan as a necessary and valuable investment
Evolution of Nepal TB Program

• Government patrol elephant deaths from TB caused concern
• Surveillance for TB in elephants in Nepal began in 2006
• Veterinary Fellowship established 2007
• Annual surveillance continued
Evolution of Nepal TB Program

• Ongoing meetings with Government
• Discussions of available diagnostics
• TB management options
  – Segregation
  – Treatment

Government of Nepal
Ministry of Forests and Soil Conservation
Department of National Parks and Wildlife Conservation
Nepal TB Plan Goals

- Eliminate TB in captive elephants and handlers
- Mitigate transmission of TB to wildlife
- Safeguard tourism
Nepal TB Plan

• All captive elephants in Nepal will be screened for TB
• Captive elephants entering Nepal from India must be screened for TB using the Elephant TB Stat-Pak® test
Nepal TB Screening Protocol

1. Perform the Elephant TB Stat-Pak® test
2. TB Stat-Pak® test non-reactive = TB free
3. TB Stat-Pak® test reactive: run DPP® VetTB™ test
4. DPP® VetTB™ test non-reactive: elephant is TB-suspect.
5. DPP® VetTB™ test reactive: elephant considered TB-infected.
Additional Points

• All elephants will be microchipped
• Private elephants licensed and registered
• Full necropsy performed on all elephants that die in Nepal
Human TB Screening Protocol

All elephant handlers and other staff working in close proximity to elephants will be screened annually for TB using free services available in Nepal.
Management Partners

• Department of National Parks and Wildlife Conservation
• National Trust for Nature Conservation
• WWF-Nepal
• Buffer Zone Management Committee
• Hotel Association Nepal
• Elephant Care International
Available Diagnostic Tools

- Culture
- Serology
Limitations of Culture

- Negative culture does not rule out disease
- Collection issues in Asian range countries
- Contamination
- Storage and transport issues
- Lab capacity / expertise in range countries
Intermittent Shedding of TB

• Sweden only 7 of 189 samples were culture positive (Moller et al. 2005)
• Thailand only 2 of 60 samples positive (Angkawanish et al. 2010)
• And…
Intermittent Shedding of TB

- elephant w proven TB exposure since 1996
- Sero-reactive since ’97
- Annual trunk washes negative
- Positive culture in 2011 within 4 weeks after daily trunk washes started

(Lyashchenko et al. 2012)
Culture does have value

- Determining drug sensitivity
- Proof of infection and shedding
- Epidemiological value for tracing
- But, more frequent testing required, and suggested by positive serology
Serology re TB in elephants

• the Elephant TB Stat-Pak® test, a screening tool with predictive values (USDA licensed 2007)

• DPP VetTB Assay – a new generation test for rapid point-of-care TB serodiagnosis in elephants (USDA licensed 2013)
Serology and WHO

• Critics* lump WHO serology comments with praise of India * Riddle, Miller, Schmitt 2012
• And no bovine TB serology programs (yet?)
• But, serology, like other tests, is validated for specific species
• Would you rely on the TST for elephants just because it works in humans and bovine?
Values of Serology

• Early predictor of infection
• More timely intervention to protect elephants, handlers, other exhibit animals, and facility reputation
• Signal to intensify trunk washes (daily), segregate, or treat, before disease progresses
Why is this so important?

• Just look at recent news and see what happens without an effective plan...
• And how it could have been different
Australian Zoo

- 2006 Taronga Zoo imported 5 Asian elephants
- In April 2009 zoo began using the Stat-Pak® test and in February 2010, the DPP
- Both tests were reactive for one elephant
- Banked serum from 2004 on the same elephant was also reactive
- A second cow seroconverted in 2011 and was treated
Oregon Zoo

• June 2, 2013 zoo announced male elephant TB culture positive
• Prior annual trunk washes negative
• Subsequently Packy, another male, reacted to serology test
• At least 2 staff reacted on TST
• Could earlier sero-positive results have reduced risk to staff and other elephants?
To care for elephants...

We should continue to develop TB management plans for captive elephants in range countries to mitigate the transmission of TB to the wild, where treatment would be impossible and to control the spread of TB amongst valuable captive elephants.
Serology Has Value

We should use all of the tools available to us...
Acknowledgements

• We greatly acknowledge the financial support of the U.S Fish and Wildlife Services Asian Elephant Conservation Fund (Awards 98201-8-G571, 96200-9-G222, and 96200-0-G143)

• And...
Acknowledgements

- USFWS
- Mazuri Fund (AAZV)
- Ernst Foundation
- AVMA Foundation
- Ocean Park Foundation
- Tulsa Zoo
- Columbus Zoo
- Busch Gardens Tampa
- Disney’s Animal Kingdom
- Dallas Zoo
- Oklahoma City Zoo
- Phoenix Zoo
- Buttonwood Park Zoo (AAZK Chapter)
- Humane Society of the United States
Our Partners in Nepal

• Department of National Parks and Wildlife Conservation
• National Trust for Nature Conservation
• WWF-Nepal
TESTING FOR TUBERCULOSIS IN ELEPHANTS: WHAT IS THE EVIDENCE?

David Miller, DVM, DACZM, PhD
Ramiro Isaza, DVM, MS, MPH, DACZM
Dennis Schmitt, DVM, PhD DACT
Jared Taylor, DVM, MPH, PhD, DACVIM, DACVPM
David Claborn, MS, DrPH
Kay Backues, DVM, DACZM
Overview

- Background

- Systematic review of diagnostic assays for tuberculosis in elephants

- Diagnostic decision-making
Elephant Tuberculosis Challenges

- Bacteria
  - *Mycobacterium tuberculosis*
  - *M. bovis*
  - Intracellular
  - Granuloma/wall off
  - Slow growing/chronic

- Human concerns

- Livestock concerns
Elephant Tuberculosis Challenges

- Clinical - general
  - Respiratory disease
  - Gastrointestinal disease
  - Wasting

- Clinical - elephants
  - Respiratory disease
  - Gastrointestinal disease
    - Wasting
    - No signs
Tuberculosis Overview

- Diagnosis – The challenge
  - Post-mortem
    - Gross
    - Microscopic
    - Culture
    - Molecular (gene probes)
Tuberculosis Overview

- Diagnosis – The challenge
  - Post-mortem

  **Ante-mortem**
  - Trunk wash + culture
    - Direct: identifies the *Mycobacterium* bacteria
    - Limitations
      - Intermittent detection (false negative)
        - Not there
        - Too few to grow and detect in culture
        - Handling of the sample
        - Problems culturing in the lab
Tuberculosis Overview

- Diagnosis – The challenge
  - Post-mortem
  - Ante-mortem
    - Trunk wash + culture

- Serology (blood test) (STAT-PAK™, MAPIA™, DPP®)
  - Indirect: identification of immune system response to TB

Limitations - source of controversy
  - Validation
    - False negatives
    - False positives
Resolution of differing opinions

Systematic review

- Challenges for elephant TB
  - Few studies
  - Limited quality
  - Qualitative assessment
Resolution of differing opinions

Solution
- Several different grading methods
- Not mutually exclusive
- Clarification of points of disagreement
Systematic Review

- Results
  - Mikota (2001): Intradermal tuberculin
    - Se: 16.7% (0.9–63.5%)
    - Sp: 74.2% (55.1–87.5%)
  - Additional tests
Systematic Review

- Study evaluations – Results
  - Data in prep

Concepts → Details
Systematic Review

- Why the variation in serologic test accuracy?
Systematic Review

- All populations are not necessarily alike
  - Test performance varies by (sub)population
Systematic Review

- What is the question: objectives for testing vary
Potential for monitoring clinical course

- Lyashchenko, et al 2012
- Serial serology
- Does not demonstrate “Predictive value”
What is the question: objectives vary

- Multiple targets of concern with differing test needs
  - Elephants – individuals & “contacts”
  - Public
  - Occupational health
Concept of analytical vs. clinical test validity

- Confusion: hierarchical assessment of diagnostic test
  - Phase I: Do “sick” and “normal” individuals have different test results?
    - Known diagnosis $\rightarrow$ diagnostic test
  - Phase II: Do test results correspond to disease likelihood?
    - Se, Sp, PV+, PV-
    - Diagnostic test result $\rightarrow$ diagnosis
    - Requires full-spectrum of disease or specify subpopulation
  - Phase III: Does test distinguish + & - among suspects?
    -Validity threatened if reference standard is lost, not done, or indeterminate

Haynes & You, 2009
Concept of analytical vs. clinical test validity

- Confusion: hierarchical assessment of diagnostic test

Evidence-based clinical decision-making

- Phase IV: Do patients receiving the test ultimately have better outcomes than patients that don’t?
  - Randomization

- Phase V: Does use of the diagnostic test lead to better health outcomes at an acceptable cost?
  - Randomization
  - External validity threatened if study subjects differ from those in “real practice”
Diagnostic Decision-Making

- Why does this matter?
Prostate cancer
- #2 cancer in men world-wide
- 6th leading cause of death in men
- More common in 1st degree relatives with prostate cancer
- Rarely has reliable early warning signs
- Usually does not cause clinical signs or symptoms

Clinician perspective: increased vigilance for screening
Is this the correct response?
Screening (PSA) for prostate cancer

- No decrease in mortality
- False-positives
  - Harms – frequent and moderate
    - Minor: bleeding, anxiety,...
    - Major: over-diagnosis and overtreatment, infection, pneumonia
  - Insufficient data available on quality of life
- Harm > benefits

Ilic et al, 2013
Relevance to elephants:
- Emotional attachment
- Un-established testing benefit
  - Risk of false-positives
    - Does the test benefit elephants?
    - Costs?
    - Risks?
Effect of disease prevalence on test accuracy

- False-positives increase as disease prevalence ↓; disease eradication programs’ challenge
  - Basic veterinary epidemiology
  - Positive predictive value rapidly declines as the prevalence of disease decreases below 10%
Key take home messages:
- Currently available research with limited external validity
- Current research in early phases of test development
- Substantial study design challenges for rigor
- Data is limited for rigorous clinical decision-making
Alphabet Soup: MICs, PK, BP, mTB and Elephants

Rob Hunter, MS, PhD
IEF Scientific Advisor
Definitions

MIC – minimum inhibitory concentration; the antimicrobial concentration, *in vitro*, at which bacterial growth in culture is inhibited for a period of 18 - 24 h (lab dependent)

PK – pharmacokinetics ($C_{max}$, AUC)

BP – break points ($S$, $I$, $R$)

mTB - tuberculosis
Introduction

- Lack of approved agents
- Limited pharmacokinetic, drug metabolism, efficacy, or toxicity data
Animal Medicinal Drug Use Clarification Act of 1994

Allows veterinarians to prescribe pharmaceutical agents in an extralabel manner
AVMA Veterinarians Oath

"Being admitted to the profession of veterinary medicine, I solemnly swear to use my scientific knowledge and skills for the benefit of society through the protection of animal health and welfare, the prevention and relief of animal suffering, the conservation of animal resources, the promotion of public health, and the advancement of medical knowledge."
Antimicrobial Therapy

Antimicrobial   Pathogen

Drug disposition → Host → Body defense mechanisms

Side effects → Host → Infection

Drug → Resistance → Organism death

Organism death → Organism
PK/PD Data

- **Pharmacokinetic Data**
  - $T_{\text{max}}$
  - $C_{\text{max}}$
  - AUC
  - $t_{1/2}$
  - $V_d$

- **Pharmacodynamic (PD) Data**
  - PK value associated with efficacy
    - $T>MIC$
    - $\text{AUC/MIC}$
Antimicrobial PK/PD Concepts

- $C_{\text{max}}$
- AUC/MIC – combined approach
- AUC
- MIC
- Time Above MIC

Concentration
ANTIMICROBIAL SUSCEPTIBILITY TESTING OF VETERINARY BACTERIAL PATHOGENS - The CLSI Perspective

CLSI Veterinary Antimicrobial Susceptibility Testing subcommittee (VAST)
What is CLSI?

Clinical and Laboratory Standards Institute (www.clsi.org)

- Standards and guidelines writing organization
  - Microbiology
  - Lab Safety
  - Hematology
  - Parasitology
  - Virology
  - Clinical Chemistry
The CLSI Process

- Tripartite participation
  - Professions (academia)
  - Government (regulatory, research)
  - Industry (pharma, manufacturers, private labs)

- Consensus process
  - Means more than agreement
    - All parties have opportunity to review and comment on documents
    - Assurance that comments will be given serious, competent consideration
CLSI Hierarchy

- Board of Directors
- Area Committee on Microbiology
  - AST Subcommittee (human pathogens)
  - VAST Subcommittee (veterinary pathogens)
    - Working Groups within each subcommittee address various topics
      - Methods
      - Reporting
      - Editorial revisions
AST & VAST Methods Relevant to mTB and Elephants

Documents for Human Pathogens
M24

Documents for Veterinary Pathogens
VET01-A4 and –S2
VET02-A3
Two Components

Quality Control & Methods

- Standardized procedures
- Quality control guidelines
- Inter- and intra-laboratory reproducibility

MIC

Agar Disk Diffusion

Interpretive criteria list

Host/pathogen/drug specific
Interpretive Categories

- **Human Pathogens***: FDA, AST
- **Veterinary Pathogens**: VAST
- **Zoonotic Pathogens***: FDA, AST
- **Indicator Bacteria***: FDA, AST

*Note: methods apply to genus-species testing irrespective of isolate origin

*FDA/CDER now sets official breakpoints; AST confirms or proposes changes to FDA.*
Interpretive Criteria for Veterinary ASTs

- Categorization of isolates as Susceptible, Intermediate, Resistant (S,I,R)
  - MIC or Agar Disk Diffusion
  - IC should be suggestive of clinical efficacy
- Development of product-specific IC
  - Host/pathogen/drug label indications and use
Susceptible: a category that implies that an infection due to the isolate may be appropriately treated with the dosage regimen of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise indicated.
Resistant: Isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or fall in the range where specific microbial resistance mechanisms are likely (e.g., β-lactamases), and clinical efficacy has not been reliable in treatment studies.
Intermediate: originally designed as a buffer zone to prevent day-to-day variations from changing susceptibility test results.

* indicates that the drug may be useful in those situations where very high drug concentrations can be achieved.
### Breakpoints for select anti-tuberculous drugs used in elephants

<table>
<thead>
<tr>
<th>Agent</th>
<th>BP concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7H10 agar</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>0.2</td>
</tr>
<tr>
<td>Rifampin</td>
<td>1.0</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>NR</td>
</tr>
<tr>
<td>Levofloxacain</td>
<td>1.0</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.5</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>2.0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

NR=not recommended; ND=not determined. Where multiple values are provided, the second is when resistance has occurred and the drugs are used as “second-line therapies” (modified from M24-A2; CLSI, 2011)
KEY POINT TO REMEMBER

KEEP IN MIND THAT “S” IS INTENDED TO HELP CLINICIANS AVOID THERAPEUTIC FAILURE. IT IS NOT INTENDED TO PREDICT THE LIKELIHOOD OF CLINICAL SUCCESS!
Extrapolation of susceptibility data from one animal to a group is dangerous.

Use susceptibility data and clinical response to evaluate therapy choices.

Pay attention to susceptibility changes in your cases within a herd/flock and between herds/flocks.

1 dilution of the MIC is within the error range of these tests.

Antimicrobials will look there best in susceptibility tests.
Determine dose, route, frequency

Pharmacokinetics

What is needed to design a dosage regimen

- \( C_p \) - target concentration
- \( V_d \)
- \( k_{el} \) or \( \beta \) - rate constants
- \( \tau \) - dosage interval

Do we have any idea if it gets to the site of infection in the species being treated?
PK studies in elephants have not evaluated necessary blood concentrations needed for *cure*, only the dose that needs to be administered to achieve blood concentrations similar to those reported in humans.

Data suggests that human BPs are likely toxic targets for elephants.

*S* is the interpretive test category implying that the infection due to the isolate may be effectively treated with the normal dosage regimen (currently unknown for elephants) of an antimicrobial agent approved for that type of infection and causative bacterial species.

**Take-aways**
Results reported by lab indicate that the isolate is “S/I/R” when tested against the diagnostic lab’s standard array of antimicrobial agents.

The diagnostic lab does not know, in the vast majority of cases, the host species, route of administration, or pharmacokinetics of the antimicrobial agent in elephants. They only have two pieces of the puzzle: microorganism and antimicrobial agent tested!
Questions?
Factors Associated with TB Transmission in Humans: Relevance in the Elephant Population

Jennifer Furin, MD., PhD.
Assistant Professor of Medicine
TB Research Unit
Case Western Reserve University
Approaches to TB

Public Health

• Control the spread
• Sensitive tests
• Maximum precautions

Patient-Centered

• Protect individual patient
• Specific tests
• Best outcome possible without exposing to unnecessary risks
Stages of TB in Humans

- Exposure
- Infection
- Active Disease

- Only humans with active TB disease are capable of transmitting TB to others
- Primary mode of transmission to humans is aerosol*
# LTBI vs. TB Disease

<table>
<thead>
<tr>
<th>Latent TB Infection (LTBI)</th>
<th>TB Disease (in the lungs)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inactive</strong>, contained tubercle bacilli in the body</td>
<td><strong>Active</strong>, multiplying tubercle bacilli in the body</td>
</tr>
<tr>
<td>TST or blood test results usually positive</td>
<td>TST or blood test results usually positive</td>
</tr>
<tr>
<td>Chest x-ray usually <strong>normal</strong></td>
<td>Chest x-ray usually <strong>abnormal</strong></td>
</tr>
<tr>
<td>Sputum smears and cultures <strong>negative</strong></td>
<td>Sputum smears and cultures may be <strong>positive</strong></td>
</tr>
<tr>
<td><strong>No symptoms</strong></td>
<td><strong>Symptoms</strong> such as cough, fever, weight loss</td>
</tr>
<tr>
<td><strong>Not infectious</strong></td>
<td><strong>Often infectious</strong> before treatment</td>
</tr>
<tr>
<td><strong>Not a case</strong> of TB</td>
<td><strong>A case</strong> of TB</td>
</tr>
</tbody>
</table>
Diagnostics in Humans

• Exposure = clinical history
• Infection = TST or IGRA or serology
• Disease, smear, culture, rapid molecular diagnostics (i.e. XpertMTB/RIF)
Transmission Factors

• Host
• Environmental

• Humans is almost always airborne (exceptions: congenital TB, *M. bovis*.)
Host Factors

- Site of disease (cougher)
- Bacillary burden (cougher)
- TB Strain (cougher)
- Immune status (exposed)
- Nutritional status (exposed)
- Genetics (both)

- Basu and Galvani, 2008, Epi and Infection
Environment

- Type of exposure
- Duration of exposure
- Ventilation
- Smoking
- Crowding

- Shenoi et al., 2010, Clin ID
TB is transmitted by aerosols (NOT sputum)
Particle size* & suspension in air

- **Particle size & deposition site**
  - 100 µ
  - 20 µ
  - 10 µ – upper airway
  - 1 - 5 µ – alveolar deposition

- **Time to fall the height of a room**
  - 10 sec
  - 4 min
  - 17 min
  - Suspended indefinitely by room air currents

*NOT organism size

from Sol Permutt, 2004
# Estimates of Mtb Aerosol Production (quanta per hour)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Quanta per hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB ward: pt on Rx</td>
<td>1.25</td>
</tr>
<tr>
<td>Cavitary TB: no Rx</td>
<td>13</td>
</tr>
<tr>
<td>Laryngeal TB</td>
<td>60</td>
</tr>
<tr>
<td>Bronchoscopy/ETT</td>
<td>250</td>
</tr>
<tr>
<td>Autopsy</td>
<td>1000</td>
</tr>
</tbody>
</table>

- Fennelly KP. Int J Tuberc Lung Dis 1998; 2: S103
Who is Infectious?

• Sputum smear + > smear –
  – AFB 3-4+ > AFB 1-2+
• Cavitary > non-cavitary
• Close > casual contact
• Prolonged > brief contact
• Men > women
• Young > old
  • Borgdorff MW et al. Am J Epidemiol 2001; 154:934
• HIV+ = HIV –
  • Cruciani M et al. Clin Infect Dis 2001; 33:1922
Where are Patients Most Infectious?

- Congregate settings
  - Hospitals
  - Correctional facilities
  - Bars
  - Choirs
  - Airplanes, ships

- Indoors >> outdoors
  - Increased with crowding & proximity
  - But no data on UV-A or UV-B effects
When are Patients Most Infectious?

- Coughing > Singing > Talking

- Aerosol producing procedures: intubation, bronchoscopy, sputum induction
  - Sepkowitz KA. Clin Infect Dis 1996;23:954

- Not on treatment
  - Unrecognized/undiagnosed
  - Drug-resistant on standard therapy
Summary

• The most infectious TB patients are those who are not on appropriate therapy
  – Undiagnosed, i.e., unrecognized
  – Drug resistant

• TB is transmitted by aerosols
  – Coughing and bacillary load important
  – Healthier patients may be more infectious

• Poorly ventilated indoor environments the highest risk
WHO/H. Darwish WHO is calling for countries to ban the use of blood tests to diagnose active TB after evidence shows the results are inaccurate. Pictured is a patient at a TB ward in Jordan undergoing a blood test. 20 July 2011 | Geneva | WHO has called for countries to ban the use of serological (blood) tests to diagnose active TB disease in a policy issued today, which described the results from these blood tests as inaccurate and a major risk to the health of patients.

Despite the wide use of these blood tests, evidence reviewed by a WHO expert group and published today concluded that "commercial serological tests provide inconsistent and imprecise estimates" and that "it is strongly recommended that these tests not be used for the diagnosis of pulmonary and extra-pulmonary TB."
Infectiousness and Diagnosis

- In order to be infectious, shedding of mycobacteria must be taking place
- Key issue will be to find animals who are shedding
- Molecular diagnostics are a new option that should be explored
Thank you!