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
HOBBLES / CHAINS
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 / 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 abscesses
• 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** for 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.
AND LET’S THANK THESE PEOPLE FOR THEIR ATTENTION OK... OK GUYS, THANKS!
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
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
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

Graph showing the relationship between B/Bo and ng/mL for both Standard and Pooled urine samples.
There were no differences ($P=0.16$) in background concentrations (0.76 ± 0.01 ng/mg Crt; 0.13 ± 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

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**Comments**

__________________________  ___________________________
Observer:                     Code/Date:
Pool usage over 2 hour period

- off exhibit
- night access
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

[Graph showing iron levels over age for males and females, with statistical information: 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

tolerated

probability

deleterious

conserved highly conserved
unconserved

SIFT calculates conservation value at each amino acid position
SIFT predicts effect of substitution at a particular position

3 candidate mutations identified in black rhinos

<table>
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<tr>
<th>Gene</th>
<th>Protein Function</th>
<th>Link to Black Rhino Phenotype</th>
<th>Mutation</th>
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<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&lt;br&gt;Q - Glutamine&lt;br&gt;K - Lysine</td>
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<tr>
<td>EPB41</td>
<td>Protein 4.1; structural element of erythrocyte membrane skeleton</td>
<td>Hemolytic anemia</td>
<td>G111E&lt;br&gt;G - Glycine&lt;br&gt;E - Glutamic acid</td>
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<tr>
<td>STEAP4</td>
<td>Six-transmembrane epithelial antigen of the prostate protein family&lt;br&gt;obesity related insulin resistance and inflammatory processes</td>
<td>Suggested link elevated iron stores and insulin resistance</td>
<td>I433S&lt;br&gt;I - Isoleucine&lt;br&gt;S - Serine</td>
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</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
Black
White
Indian
Sumatran
horse
opossum
deer
pig
sheep
yak
bat
elephant
manatee
walrus
panda
dog
cat
mouse
rat
hamster
gorilla
human
baboon
chimp
bonobo
rabbit
marmoset
squirrel_monkey
armadillo
rhesus
bushbaby
tree_shrew
turtle
dolphin
orca
lizard
finch
mallard
tasmanian_devil
gibbon
mole_rat
guinea_pig

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<tr>
<td>R</td>
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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
Sumatran
Indian
White
horse
platypus
gibbon
gorilla
chimp
human
rheus
baboon
squirrel_monkey
marmoset
elephant
manatee
lizard
mole_rat
bushbaby
trogdor
walrus
panda
dog
cat
dolphin
orca
sheep
cow
yak
frog
clawed_frog
opossum
mallard
mouse
armadillo
bat
pufferfish
ricefish
tilapia
rabbit
turkey
chicken
finch
guinea_pig
pig
rat
hamster

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<tr>
<td>V Valine</td>
<td>Hydrophobic</td>
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<tr>
<td>M Methionine</td>
<td>Hydrophobic</td>
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Not a conservative substitution
Position STEAP4 black rhino mutation

- STEAP4 I433S located in the α7 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

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, Myanma 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 Myanma 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.
Total Elephant population

In MTE - 2861 (Based on 2013 MTE record)
In Private - < 2000 (Based on FD record)
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%
- Elephant Attack: 6%

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

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 thanks 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 straightforward
- 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?

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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

[Image of Mycobacterium tuberculosis]

[Image of elephant lung tissue]
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
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
Systematic Review

- 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
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
Systematic Review

- 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
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

<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>
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</tr>
</tbody>
</table>

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

\[
\text{Graph A}_{\infty}
\]

\[\text{TB Antibody Level} \quad 0 \]

\[\text{Number of elephants} \quad 0\]

\[\text{Stage of Infection} \quad \text{Uninfected elephants} \]

\[
\text{Time} \quad \text{Increasing bacteria number} \quad \text{Increasing host pathology}
\]

\[
\text{Subclinical/early infections?} \quad \text{Late stage infected elephants}
\]

\[
\text{Early stage infections} \quad \text{Late stage infections}
\]
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”

<table>
<thead>
<tr>
<th>Test Positive</th>
<th>Disease Positive</th>
<th>Disease Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Positive</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Test Negative</td>
<td>c</td>
<td>d</td>
</tr>
</tbody>
</table>
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
Systematic Review

- 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
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?
Diagnostic Decision-Making

- 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

Diagnostic Test with 80% Sensitivity and 80% Specificity

Diagnostic Test with 60% Sensitivity and 60% Specificity

<table>
<thead>
<tr>
<th>True prevalence of disease = 5%</th>
<th>With validated test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PV+</td>
</tr>
<tr>
<td>Se 95%/Sp 95%</td>
<td>50%</td>
</tr>
<tr>
<td>Se 80%/Sp 80%</td>
<td>17%</td>
</tr>
<tr>
<td>Se 60%/Sp 60%</td>
<td>7%</td>
</tr>
</tbody>
</table>
Diagnosis 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

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**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

<table>
<thead>
<tr>
<th>ID</th>
<th>Year</th>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>Status</th>
<th>Captured</th>
<th>Death</th>
<th>Transfer</th>
<th>Birth</th>
<th>Outgoing</th>
</tr>
</thead>
<tbody>
<tr>
<td>497</td>
<td>2026</td>
<td>100497</td>
<td>497S</td>
<td>1</td>
<td>Asian</td>
<td>WILD</td>
<td>WILD</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>498</td>
<td>2026</td>
<td>100498</td>
<td>498S</td>
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<td>WILD</td>
<td>WILD</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- **ID**: Unique identifier for each elephant.
- **Year**: Year of the data entry.
- **Name**: Name of the elephant.
- **Age**: Age of the elephant.
- **Sex**: Gender of the elephant.
- **Status**: Current status of the elephant (WILD, Transfer, Death, etc.).
- **Captured**: Whether the elephant was captured (0 or 1).
- **Death**: Whether the elephant died (0 or 1).
- **Transfer**: Whether the elephant was transferred (0 or 1).
- **Birth**: Whether the elephant was born (0 or 1).
- **Outgoing**: Whether the elephant left the facility (0 or 1).
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

<table>
<thead>
<tr>
<th>Measures of M. tb complex for both species of elephants</th>
</tr>
</thead>
<tbody>
<tr>
<td>- What is the annual point prevalence?</td>
</tr>
<tr>
<td>- What is the annual incidence?</td>
</tr>
<tr>
<td>- What is the incidence density?</td>
</tr>
</tbody>
</table>
**Introduction**

**Mandatory math review**

\[
\frac{3}{4} \quad \text{Numerator}
\]

\[
\text{Elephant M. tb “Cases”}
\]

\[
\text{Elephant Population *}
\]

* 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)
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
Point prevalence of \textbf{M. tb} complex from 1960 to 2011

- Calculated on the first day of each year
M. tb Complex in Asian Elephants

PREVALENCE OF TUBERCULOSIS IN ASIAN ELEPHANTS

Prevalence (%)

Year

<table>
<thead>
<tr>
<th>Prevalence of <em>M. tb</em> complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median since 1997 = 5.0%</td>
</tr>
<tr>
<td>Range = 1.2 – 5.8%</td>
</tr>
</tbody>
</table>
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
Methods for Incidence

- Incidence of elephants culture positive for *M. tb* complex from 1960 to 2011
  - Calculated for each year

\[
\frac{\text{Number of new cases in one year}}{\text{Average population during that year}} = \text{Annual Incidence}
\]
M. tb Complex in Asian Elephants

NEW CASES OF TUBERCULOSIS IN ASIAN ELEPHANTS

Total New Cases

Year

M. tb Complex in African Elephants

NEW CASES OF TUBERCULOSIS IN AFRICAN ELEPHANTS

Year

Total New Cases

0 1 2 3 4 5 6 7 8 9 10

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
**African Elephant Incidence Density**

<table>
<thead>
<tr>
<th>Incidence Density of <strong>M. tb</strong> complex</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Annual incidence density between 1997 and 2011</strong></td>
</tr>
<tr>
<td>1.5 cases /1000 elephant years</td>
</tr>
<tr>
<td>(95%:0.7-4) since 1998</td>
</tr>
</tbody>
</table>
Conclusions

**M. tb complex in elephants**
- Need accurate census data
- Need well defined cases and populations
- Need consistent calculations
**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