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
3rd Annual Management and Research Priorities of Tuberculosis for Elephants in Human Care Workshop (ECT)
August 25-26th, 2013
Pittsburgh, PA, USA.

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

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

10:15 – 10:30  Break

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
<table>
<thead>
<tr>
<th>PARTICIPANT NAME</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>David Abraham</td>
<td>Centre for Ecology and Wildlife Diseases</td>
</tr>
<tr>
<td>Tom Albert</td>
<td>Feld Entertainment/RBBB</td>
</tr>
<tr>
<td>Jill Allread - facilitator</td>
<td>Public Communications Inc.</td>
</tr>
<tr>
<td>Doug Armstrong</td>
<td>American Association of Zoo Veterinarians</td>
</tr>
<tr>
<td>Kay Backues</td>
<td>American Association of Zoo Veterinarians</td>
</tr>
<tr>
<td>Colin Basler</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>Mitch Finnegan</td>
<td>Oregon Zoo</td>
</tr>
<tr>
<td>Martha Fischer</td>
<td>St. Louis Zoo</td>
</tr>
<tr>
<td>Jennifer Furin</td>
<td>Case Western Reserve University</td>
</tr>
<tr>
<td>Joan Galvin</td>
<td>Kelley Drye &amp; Warren LLP</td>
</tr>
<tr>
<td>Matt Gombrich</td>
<td>Vivian biosciences</td>
</tr>
<tr>
<td>Hank Hammatt</td>
<td>Elephant Care International</td>
</tr>
<tr>
<td>Rob Hunter</td>
<td>International Elephant Foundation</td>
</tr>
<tr>
<td>Ramiro Isaza</td>
<td>University of Florida</td>
</tr>
<tr>
<td>Kari Johnson</td>
<td>Have Trunk Will Travel</td>
</tr>
<tr>
<td>Bob Lee</td>
<td>Oregon Zoo</td>
</tr>
<tr>
<td>AJ Marlar</td>
<td>Fort Worth Zoo</td>
</tr>
<tr>
<td>Joel Maslow</td>
<td>University of Pennsylvania</td>
</tr>
<tr>
<td>Mike McClure</td>
<td>Elephant Managers Association</td>
</tr>
<tr>
<td>Bob Meyer</td>
<td>National Assembly of State Veterinarians</td>
</tr>
<tr>
<td>Corissa Miller</td>
<td>Ecotone Wildlife Veterinary Services</td>
</tr>
<tr>
<td>Dave Miller</td>
<td>Independent</td>
</tr>
<tr>
<td>Michele Miller</td>
<td>Rare Species Conservatory Foundation</td>
</tr>
<tr>
<td>Julia Murphy</td>
<td>American Association of Public Health Veterinarians</td>
</tr>
<tr>
<td>Debbie Myers</td>
<td>Pittsburgh Zoo</td>
</tr>
<tr>
<td>Meenakshi Nagendran</td>
<td>United States Fish and Wildlife Service</td>
</tr>
<tr>
<td>Dustin Oedekoven</td>
<td>United States Animal Health Association</td>
</tr>
<tr>
<td>Francisco Olea-Popelka</td>
<td>Colorado State</td>
</tr>
<tr>
<td>Florence Olivet-Courtois</td>
<td>médecine des animaux sauvages et exotiques</td>
</tr>
<tr>
<td>Name</td>
<td>Organization</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Deborah Olson</td>
<td>International Elephant Foundation</td>
</tr>
<tr>
<td>Janet Payeur</td>
<td>United States Animal Health Association</td>
</tr>
<tr>
<td>Heidi Riddle</td>
<td>Elephant Managers Association</td>
</tr>
<tr>
<td>Dennis Schmitt</td>
<td>Feld Entertainment/RBBB</td>
</tr>
<tr>
<td>Denise Sofranko</td>
<td>USDA/APHIS/Animal Care</td>
</tr>
<tr>
<td>Christopher Stremme</td>
<td>Veterinary Society for Sumatran Wildlife Conservation</td>
</tr>
<tr>
<td>Ginger Takle</td>
<td>Pittsburgh Zoo</td>
</tr>
<tr>
<td>Ellen Wiedner</td>
<td>University of Florida</td>
</tr>
<tr>
<td>Erica Wilson</td>
<td>Dickerson Park Zoo</td>
</tr>
<tr>
<td>Mark Wilson</td>
<td>Florida International Teaching School</td>
</tr>
<tr>
<td>Ralph Zimmerman</td>
<td>Albuquerque Biopark</td>
</tr>
</tbody>
</table>
WORKSHOP STATEMENT

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.tbc* 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
  o State public health vet
  o State veterinarians
  o 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
  - Transporting of elephants
  - Potential risk exposure/transmission to livestock
  - Transport of elephants, especially movement of elephants that might be exposed but not monitored (tested)
  - Public health risk
- Regulatory agencies
- Government
- Elephant community
- General public
- Facility staff
- Administrators
- Colleagues
Media
Extremists

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.pinnipedi
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/)
SOME CONSIDERATIONS ON MYCOBACETRIUM TUBERCULOSIS IN ELEPHANTS IN ASIAN RANGE COUNTRIES

Christopher Stremme
Veterinary Society for Sumatran Wildlife Conservation
SUMATRA / INDONESIA
SUMATRA / INDONESIA
POST MORTEMS
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

<table>
<thead>
<tr>
<th>PCR positive for $M.\ tuberculosis$ and $M. bovis$</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (marker 100 bp)</td>
</tr>
<tr>
<td>kolom 1 (Kidney)</td>
</tr>
<tr>
<td>kolom 2 (Lung)</td>
</tr>
<tr>
<td>kolom 3 (Liver)</td>
</tr>
<tr>
<td>kolom 4 ($M.\ tuberculosis$)</td>
</tr>
<tr>
<td>kolom 5 ($M. bovis$)</td>
</tr>
<tr>
<td>kolom 6 (nuclease free water)</td>
</tr>
</tbody>
</table>
### 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>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>660,000</td>
<td>1,000,000</td>
<td>99,000</td>
<td>608,628,000</td>
</tr>
<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>
</tr>
<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>
</tr>
<tr>
<td>Global Total</td>
<td>8,800,000</td>
<td>11,830,000</td>
<td>995,000</td>
<td>6,947,687,000</td>
</tr>
</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>
</tr>
</thead>
<tbody>
<tr>
<td>Afghanistan</td>
<td>61,000</td>
<td>110,000</td>
<td>13,000</td>
<td>32,358,000</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>340,000</td>
<td>620,000</td>
<td>68,000</td>
<td>150,494,000</td>
</tr>
<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>
</tr>
<tr>
<td>China</td>
<td>1,000,000</td>
<td>1,400,000</td>
<td>47,000</td>
<td>1,347,565,000</td>
</tr>
<tr>
<td>DR Congo</td>
<td>220,000</td>
<td>350,000</td>
<td>36,000</td>
<td>67,758,000</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>220,000</td>
<td>200,000</td>
<td>15,000</td>
<td>84,734,000</td>
</tr>
<tr>
<td>India</td>
<td>2,200,000</td>
<td>3,100,000</td>
<td>300,000</td>
<td>1,241,492,000</td>
</tr>
<tr>
<td>Indonesia</td>
<td>450,000</td>
<td>680,000</td>
<td>65,000</td>
<td>242,326</td>
</tr>
<tr>
<td>Kenya</td>
<td>120,000</td>
<td>120,000</td>
<td>9,200</td>
<td>41,610</td>
</tr>
<tr>
<td>Mozambique</td>
<td>130,000</td>
<td>120,000</td>
<td>11,000</td>
<td>23,930,000</td>
</tr>
<tr>
<td>Myanmar</td>
<td>180,000</td>
<td>240,000</td>
<td>23,000</td>
<td>48,337,000</td>
</tr>
<tr>
<td>Nigeria</td>
<td>190,000</td>
<td>280,000</td>
<td>27,000</td>
<td>162,471,000</td>
</tr>
<tr>
<td>Pakistan</td>
<td>410,000</td>
<td>620,000</td>
<td>59,000</td>
<td>176,745,000</td>
</tr>
<tr>
<td>Philippines</td>
<td>260,000</td>
<td>460,000</td>
<td>28,000</td>
<td>94,852,000</td>
</tr>
<tr>
<td>Russian Federation</td>
<td>140,000</td>
<td>180,000</td>
<td>22,000</td>
<td>142,836,000</td>
</tr>
<tr>
<td>South Africa</td>
<td>500,000</td>
<td>390,000</td>
<td>25,000</td>
<td>50,460,000</td>
</tr>
<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>
</tr>
<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>
</tr>
<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.

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.
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.
SRI LANKA

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>Ocular disease</td>
<td>55</td>
<td>9</td>
<td>Poisoning</td>
<td>46</td>
<td>8</td>
</tr>
<tr>
<td>Foot pathology</td>
<td>48</td>
<td>8</td>
<td>Old-age related</td>
<td>44</td>
<td>7</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>44</td>
<td>7</td>
<td>Nonspecific</td>
<td>39</td>
<td>7</td>
</tr>
<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>
</tr>
<tr>
<td>Skin disease</td>
<td>7</td>
<td>1</td>
<td>Cardiac disease</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Stereotypical behavior</td>
<td>5</td>
<td>1</td>
<td>Hemorrhagic disease</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Over work</td>
<td>4</td>
<td>1</td>
<td>lack of veterinary care</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Poisoning</td>
<td>4</td>
<td>1</td>
<td>Lameness</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Renal disease</td>
<td>4</td>
<td>1</td>
<td>Musth</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Cancer</td>
<td>3</td>
<td>0.5</td>
<td>Respiratory disease</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Ventral edema</td>
<td>3</td>
<td>0.5</td>
<td>Dehydration</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Drug reaction</td>
<td>1</td>
<td>0.2</td>
<td>Prolonged recumbency</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Reproductive problem</td>
<td>1</td>
<td>0.2</td>
<td>Seizure</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Tusk pathology</td>
<td>1</td>
<td>0.2</td>
<td>Congenital disease</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neurologic disease</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reproductive problem</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chemical immobilization</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

100
Importance of TB in relation to other diseases in Asian elephant

<table>
<thead>
<tr>
<th>REPORTED POST-MORTEM PATHOLOGIC FINDINGS</th>
<th>SUM OF SCORES</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human elephant conflict (electrocution, poisoning, poaching, train collision, gunshot, wells, pit traps, snares, etc.)</td>
<td>53</td>
<td>10.4</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>52</td>
<td>10.2</td>
</tr>
<tr>
<td>Endoparasites</td>
<td>38</td>
<td>7.5</td>
</tr>
<tr>
<td>Gastrointestinal stasis or torsion</td>
<td>34</td>
<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>
<td>21</td>
<td>4.1</td>
</tr>
<tr>
<td>Injuries from intraspecific aggression</td>
<td>20</td>
<td>3.9</td>
</tr>
<tr>
<td>Toxin</td>
<td>18</td>
<td>3.5</td>
</tr>
<tr>
<td>Splenic lesions</td>
<td>18</td>
<td>3.5</td>
</tr>
<tr>
<td>Tetanus</td>
<td>18</td>
<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>
</tr>
<tr>
<td>Traumatic injuries</td>
<td>16</td>
<td>3.1</td>
</tr>
<tr>
<td>Sepsis</td>
<td>14</td>
<td>2.7</td>
</tr>
<tr>
<td>Emaciation</td>
<td>12</td>
<td>2.4</td>
</tr>
<tr>
<td>Skin lesions</td>
<td>11</td>
<td>2.2</td>
</tr>
<tr>
<td>Rabies</td>
<td>7</td>
<td>1.4</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>7</td>
<td>1.4</td>
</tr>
<tr>
<td>Elephant entotheliotropic herpes virus</td>
<td>7</td>
<td>1.4</td>
</tr>
<tr>
<td>Autolysis</td>
<td>6</td>
<td>1.2</td>
</tr>
<tr>
<td>Cyanosis</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>Lightning</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>Salmonellosis</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>Arthritis</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>Anemia</td>
<td>3</td>
<td>0.6</td>
</tr>
<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>GRAND TOTAL</td>
<td>510</td>
<td>100</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 INFECTIONOUS 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
MEDICAL HISTORY

• 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>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ERT</strong></td>
<td>reactive</td>
<td>reactive</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td><strong>ELISA</strong></td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td><strong>MAPIA</strong></td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td><strong>DPP</strong></td>
<td>reactive</td>
<td>negative</td>
<td>reactive</td>
<td>negative</td>
</tr>
<tr>
<td><strong>DPP expérimental not validated in 2010</strong></td>
<td>reactive</td>
<td>negative</td>
<td>reactive</td>
<td>negative</td>
</tr>
</tbody>
</table>
USDA PROTOCOLE FOR THE SITUATION IN 2010

- 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
OBSTRUCTION

• The zoo refused to do new tests because the animals were dangerous,

• MAPIA negative results of 2010 not transmitted to the court and experts
PRO EUTHANASIA ARGUMENTS

- 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
• 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)
NEWS

• ANSES : « Animals should be tested » (no time limit !)
• A new sanitary facility built by princess of Monaco
• Treatment forbidden
ANSES REPORT

• « 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?
ANSES CONCLUSION ABOUT CONSERVATION

• 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
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
• 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

INTERNATIONAL ELEPHANT FOUNDATION.ORG
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**

---

http://ard.bmj.com/content/63/suppl_2/ii50.ful
Elephant Tuberculosis Challenges

- Clinical - general
  - Respiratory disease
  - Gastrointestinal disease
  - Wasting

- Clinical - elephants
  - Respiratory disease
  - Gastrointestinal disease
  - Wasting
  - No signs
Elephant Controversies
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
  - **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
Systematic Review

- 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
Study evaluations – Results
  - Data in prep

Concepts ➔ Details
Systematic Review

- Why the variation in serologic test accuracy?
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 → 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?
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 ($C_{\text{max}}$, 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
"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

Side effects → Body defense mechanisms

Infection → Drug

Organism death → Resistance

Organism
Antibiotic Selection Matrix

Antimicrobial Agent

Cost
> Cost of treatment
> Value of animal
> Loss of product

Principles of Treatment
> Pharmacokinetic considerations
  > Dosage
  > Dosage modification: neonate; renal failure; liver damage
  > Duration: acute or chronic infection
  > Site of infection
  > Nature of infection
  > Neutropenia; impaired defenses
  > Combinations

Risk
> Direct host toxicity
> Adverse drug interactions
> Destruction of normal microflora
> Promotion of drug resistance
> Tissue injection damage
> Tissue drug residues
> Impairment of host defenses?
> Fetus? > Neonate?

Microorganism
> Organism known or suspected
> Historical susceptibility
> Susceptibility test results: MIC data
> Clinical experience with type of infection
> Extracellular infection?
> Intracellular infection?

Pharmacodynamic Parameters
> MIC > MBC
> Increased killing with exposure to increased concentration
  > First exposure effect
  > Post-antibiotic effect (PAE)
  > Sub-MIC effect (SM-PAE)
  > Post-antibiotic leukocyte enhancement (PALE)

Pharmacokinetic Considerations
> Route of administration: IV, IM, SC, oral local
> Physicochemical properties of the drug
> Distribution and elimination characteristics
> Volume of distribution: half-life clearance
> Barriers to penetration
PK/PD Data

- **Pharmacokinetic Data**
  - $T_{\text{max}}$
  - $C_{\text{max}}$
  - AUC
  - $t_{\frac{1}{2}}$
  - $V_d$

- **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
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

3rd Annual Management and Research Priorities of Tuberculosis for Elephants in Human Care Workshop
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
Antimicrobial Susceptibility Testing VET01, VET02, M24

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

<table>
<thead>
<tr>
<th>Category</th>
<th>Agency(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Pathogens*</td>
<td>FDA, AST</td>
</tr>
<tr>
<td>Veterinary Pathogens</td>
<td>VAST</td>
</tr>
<tr>
<td>Zoonotic Pathogens*</td>
<td>FDA, AST</td>
</tr>
<tr>
<td>Indicator Bacteria*</td>
<td>FDA, AST</td>
</tr>
</tbody>
</table>

*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
Interpretive Categories

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 (eg, β-lactamases), and clinical efficacy has not been reliable in treatment studies.
Interpretive Categories

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>
<th>7H10 agar</th>
<th>7H11 agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td></td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Rifampin</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Ethambutol</td>
<td></td>
<td>5.0</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NR&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td></td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td></td>
<td>1.0</td>
<td>ND</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td></td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td></td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</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?
Take-aways

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.
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 negative</td>
<td>Sputum smears and cultures may be <strong>positive</strong></td>
</tr>
<tr>
<td>No symptoms</td>
<td><strong>Symptoms</strong> such as cough, fever, weight loss</td>
</tr>
<tr>
<td>Not infectious</td>
<td><strong>Often infectious</strong> before treatment</td>
</tr>
<tr>
<td>Not a case of TB</td>
<td>A case 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
- HIV+ = HIV –
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!