

**MANAGEMENT AND RESEARCH PRIORITIES OF
TUBERCULOSIS FOR ELEPHANTS IN HUMAN CARE –
STAKEHOLDERS TASK FORCE**

1-2 August 2011

**Fort Worth Zoo
Fort Worth, Texas**



Hosted by the American Association of Zoo Veterinarians, Association of Zoos and Aquariums Elephant Taxon Advisory Group, Elephant Managers Association, Fort Worth Zoo, International Elephant Foundation, Ringling Bros. Center for Elephant Conservation

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An electronic version of this report can be downloaded at www.elephantconservation.org

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WORKSHOP STATEMENT

Since 1998, the United States Department of Agriculture – Animal and Plant Health Inspection Service Animal Care (USDA-APHIS-AC) has relied upon guidelines recommended by an ad hoc committee (under the auspices of the American Association of Zoo Veterinarians) of veterinarians, researchers and epidemiologists regarding the management of *Mycobacterium tuberculosis* (MTB) complex in elephants. At that time, 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 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 requested to take responsibility for developing Guidelines for the Control of Tuberculosis in Elephants (Elephant TB Guidelines). In 2008, the USDA Elephant TB Guidelines incorporated the use of a USDA licensed serological screening test (ElephantTB STAT-PAK®, Chembio Diagnostics Inc., New Medford, NY) for TB antibodies (*M.tb* and *M.bovis*) as a complement to culture to identify “infected” elephants, and elephants at risk of developing tuberculosis or shedding organisms. In 2010, USDA-APHIS-AC incorporated these serological tests into its elephant tuberculosis regulatory program. In 2010 the USAHA subcommittee recommended changes to the Guidelines that placed greater emphasis on the ElephantTB STAT-PAK® and a confirmatory test – the Multi-Antigen Print Immunoassay (MAPIA) together with exposure history but these were not rigorously defined (based on current 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 which calls into question some 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.

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.

Management and Research Priorities of Tuberculosis for Elephants in Human Care - Stakeholders Task Force

**Facilitator – Egeenee Daniels DVM, Director of
Laboratory Animal Medicine, University of North
Texas**

Agenda

Monday August 1, 2011

| | |
|---------------------------|--|
| 8:00 am - 8:30 am | Welcome – Mike Fouraker, Kay Bacchus Participant introductions- name and affiliation/ experience with TB Brief summarization of agenda and goals |
| 8:30 am -10:00 am | Overview of Tuberculosis in elephants History of TB in elephants in North America - Susan Mikota Overview of Guidelines - Michele Miller Overview of testing - Ramiro Isaza Overview of treatment experiences - Dennis Schmitt Overview of TB and elephants from the elephant managers' perspective – Mike McClure & Heidi Riddle Questions and Discussion of material presented |
| 10:00 am -10:15 am | Coffee Break |
| 10:15 am - 10:45am | Update of previous Tuberculosis Workshop outcomes – Dennis Schmitt |
| 10:45 am – 11:30 am | Open group discussion – Additional problems and action items that need further investigation |
| 11:30 am – 12:00 pm | Review suggestions and group items into categories/Major themes |
| 12:00 pm – 12:30 am | Identify most important action items from flipchart pages |

12:00 pm -1:30 pm

Lunch

1:30 pm - 2:00 pm

Review morning outcomes, make changes/edits to high priority action items
Break out session

2:00 pm – 3:30 pm

3:30pm - 4:00 pm

Coffee Break

4:00 pm - 6:00 pm

Reconvene as one group and reports given

Tuesday August 2, 2011

8:00 am - 8:30 am

Brief summarization of agenda and goals

8:30 am -10:00 am

Break out session

10:00 am - 10:15 am

Coffee Break

10:15 am – 12:00 pm

Reconvene as one group and reports given

12:00 pm – 1:00 pm

Lunch

1:00 pm - 3:00 pm

Based on working group sessions, summarize meeting and develop consensus Workshop outcomes

3:15 pm - 3:30 pm

Coffee Break

3:30 pm – 5:00 pm

Continuation of afternoon discussion

Executive Summary

Introduction

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

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

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

As a result, in early August of 2011, *Management and Research – Priorities of Tuberculosis for Elephants in Human Care – Stakeholders Task Force* was held in Ft. Worth. This workshop was organized and hosted by the American Association of Zoo Veterinarians (AAZV), the American Association of Zoo and Aquariums (AZA) Elephant Taxon Advisory Group (TAG), the Elephant Managers Association (EMA), the International Elephant Foundation (IEF), Fort Worth Zoo and Ringling Bros Center for Elephant Conservation. Invited participants included elephant managers and veterinarians, researchers, zoo and traveling exhibitors, state public health veterinarians and human infectious disease and public health experts.

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 best practices for managing TB in elephants. Through this workshop format, participants discussed the current available knowledge, focused on sharing information, established potential areas of collaboration and common goals. They identified actionable items to improve detection, diagnosis, treatment, and ultimately to reduce the impact of this devastating disease so that we may ensure a future for captive and wild elephants.

Key goals include 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, and how those definitions can best be reflected in the development of new guidelines that are practical, realistic, and effective in controlling MTB in elephants; 4) formulating travel and risk recommendations; and 5) developing short-term and long-range goals in the areas of research, disease management, herd monitoring, herd management, public health, funding, and public relations.

Process

A structured workshop approach was taken to develop specific goals and objectives. Key projects were identified and action plans outlined for implementation with a timeline, due date, responsible individuals and a champion to oversee each goal and encourage responsible parties to stay enthused and on task. In addition, action plans allow for continuous review, identification of actions implemented and new actions to be enacted. The workshop started with an introduction explaining to the participants that the aim of this workshop was to design some key projects, small actions and a continuous improvement process in order to advance our knowledge of tuberculosis and its management in elephants.

The format of this workshop was structured to analyze issues and questions and to develop creative and inclusive solutions. Most of the workshop was spent in working groups, with occasional reports back to all participants in plenary sessions for comments and revision. Group work allows for effective and efficient use of time while plenary sessions allow all participants to have input on all workshop recommendations.

This Workshop was designed to help participants achieve the objectives listed as priority goals during the initial open group discussion. In order to ensure that all participants had a common base of understanding, the workshop agenda included overview presentations.

Ground rules were provided since time was limited. These rules included everyone must listen and respect others, avoid side conversations, no negative comments and no statement is criticized, everyone is considered equal, focus comments, be open to suggestion, share information, try to reach conceptual consensus and when everyone is comfortable with the decision, it is recorded.

Identification of Priority Goals

The group brainstormed goals/actions for addressing the issues related to MTB complex in elephants. Each comment was noted on flip charts. Participants were asked to review the goals and ensure there is a common understanding of each. The goals were then consolidated. Pages were rewritten so that each point identified could be grouped and put on the same flip chart page under a major theme. Sheets were reviewed to see if missing goals needed to be added. The group prioritized the goals to identify those they felt were most promising using colored dots. Everyone received 4 “dots” to distribute on action items they thought most important. All the dots could be placed on the same objective or put on different ones.

Top Priority Goals followed by number of votes

Treatment

- 1) Identify appropriate monitoring parameters and develop standard protocol to collect samples on a standard schedule – 6
- 2) Better define adverse effects of treatment - 6
- 3) Better define treatment protocol - 3
- 4) Identify biomarkers other than MAPIA – 2
- 5) Identify what are the most appropriate markers of treatment toxicity in elephants - 1
- 6) Repeat pharmacokinetic studies and look at different TB drugs - 1
- 7) Enact a formal retrospective study to drive future studies and data collection - 1
- 8) Produce a cost/benefit model – 1
- 9) Determine the concentration or dose—response curve for each TB prescription case
- 10) Determine the concentration or dose-toxicity curve for each TB prescription
- 11) Determine the most appropriate markers of treatment response in elephants
- 12) Design treatment trial (clinical trial)
- 13) Standardize treatment protocol form
- 14) Drug treatment – humans 6 months with Rifampin, elephants non-compliant with Rifampin
- 15) Define elephant pathology, pathophysiology, natural course of infection and disease
- 16) Pharmacology
- 17) Pharmacogenetics
- 18) Borrow from current human TB research to help guide
- 19) Develop an electronic Library of TB information

Epidemiology

- 1) Define Exposure – elephant to elephant, elephant to human, human to elephant - 14
- 2) Define Infection/disease - 12
 - a. Risk of transmission
 - b. Risk of progression
 - c. Decision analysis
- 3) Develop single, neutral, centralized data repository to enable the sharing of data and to make use of 15 years of data that has been collected -11
- 4) Develop standardized reporting form –11

- 5) Develop strategies to collect data from NVSL , on human staff and elephants (trunk wash, genetics, ElephantTB STAT-PAK®, MAPIA)
- 6) Identify current demographics of elephants in North America - gather list of positive animals, test results etc
- 7) Identify characteristics of isolates – retrospective and prospective
- 8) Centralize isolates-release to appropriate researchers
- 9) Collate cases for risk factor analysis
- 10) Improve direct detection technique ElephantTB STAT-PAK®-PCR and other tests
- 11) Identify characteristics of infected elephants (current and infected elephants)
- 12) Identify what makes an elephant susceptible to disease - -immunologic factors (MHC), environmental, other risk factors
- 13) Need to compare elephant TB with human TB – strains, contact, (N American, Europe, and range communities), bovine, cervid
- 14) Definition of TB infection and disease
- 15) Develop means to follow animals over time – help determine truly positive or truly negative
- 16) Determine what latency means
- 17) Clarify how exposure history could be useful in guidelines

Public Health Issues

- 1) Initiate an Occupational Risk study - 7
- 2) Determine practical precautions needed to work with elephants based on risk category for the group (as identified in the guidelines) - 2
- 3) Develop means to tell when an elephant is shedding TB
- 4) Measure the level of TB infection in elephant workers
- 5) Develop a proactive relationship with public health officials and regulatory agents
- 6) Provide education about elephant TB in elephant workers and related staff
- 7) Establish mandatory screening of elephant workers that is standardized and documented
- 8) Develop methods of measuring risks of specific elephant products
- 9) Measure prevalence of TB in the elephant population
- 10) Educate public health officials and health care providers about occupational risks of working with TB elephants

Immunology

- 1) Focus on MAPIA reactive and negative predictive value -4
- 2) Investigate treated versus untreated
- 3) IGM has never been in elephants – Define its role and validate
- 4) Can humoral response differentiate infection from disease

Diagnostics

- 1) Real World implications and management - 9
- 2) Additional diagnostic methods - PCR etc. - 3
- 3) 3 x per week treatment for shorter duration – 3
- 4) Research latency - can we determine if latency or clearance, could a population of positives, age and origin suggest latency - 2
- 5) Identify objectives for management of TB in elephants - 1

- 6) Clarify frequency of trunk wash cultures for MAPIA reactive animals - 1
- 7) Exposure history emphasized in guidelines - 1
- 8) Focus on MAPIA Test – reactive and negative predictive value
- 9) Clarify the MAPIA and ElephantTB STAT-PAK® tests - why do they not agree at times.
What does a reactive ElephantTB STAT-PAK® and a non-reactive MAPIA on the same elephant mean?
- 10) How can we follow animals over time to determine predictive value of these tests?
- 11) Develop pulmonary function tests
- 12) Define typical sensitivity patterns
- 13) Review guidelines
- 14) Standardized collection form for better access to data
- 15) Up to date list of positive animals for analysis

The votes were counted and the top priority objectives identified which allowed the working groups to also be identified:

1. Treatment
2. Diagnostics & Immunology
3. Occupational Health
4. Epidemiology & Data Management

Participants chose the working group in which they were most interested. Each group designated a reporter (someone who reports the group's findings during plenary) and a scribe (w/ computer if possible) who recorded all information produced by the group, and a leader to keep everyone on task. All written material were transferred to a flash drive and given to conference hosts for proceedings.

Working Groups Results

Epidemiology Working Group

| | | | |
|-------------|----------------|------------------|--------------|
| Tom Albert | Sharon Deem | Terry S. Hensley | Thomas Holt |
| Dave Miller | Michele Miller | Thaddeus Miller | Heidi Riddle |

Objectives of managing TB in elephants:

- Keep elephants alive, healthy, TB free
- Minimize the transmission of TB between elephants and elephants, and people and elephants

To determine Disease Risk Analysis:

- Hazard identification
- Risk Assessment
- Risk communication
- Risk management

Action Items:

- 1) Methods for sample sharing
- 2) Dovetail with diagnostic working group (work with established entities – AAZV, SSP, IEF), sample sharing, website posting for collaboration
- 3) Identify person(s) /team to lead epidemiologic working group
 - a. Select risk factors, diagnostics, etc to investigate
 - b. Sample sharing – banking? Collaboration at first
 - c. Apply for funding for items in (a)
 - d. Report back data
 - e. Anonymous submission
- 4) PPV/NPV for MAPIA/ ElephantTB STAT-PAK®
Already being investigated by Dr. Miller
Encourage data submission – ongoing
- 5) Agree on definition of latency for use in research and regulatory arenas
- 6) Input into data collection form so that create PPV/NPV on diagnostics
- 7) Age, gender, # trunk wash cultures/serological tests & results , exposures to culture (+) animal, when and how long, past history duration, past samples

What are the risk factors for Exposure, Infection, Disease, Progression?

| Exposure | Infection | Disease | Progression |
|------------------------------|------------------------|--------------------------|--------------------------|
| Intensity | Increasing travel | Decreasing immune status | Decreasing Immune status |
| Duration time | TB history at facility | TB treatment history | No treatment |
| Space | Signalment | Signalment | Increase stress |
| Family unit/friends | Public contact | Increase stress | Co-infection |
| Facility design | Serologic status | Serologic status | |
| TB Positive human contact | | | |
| TB Positive elephant contact | | | |
| Importation | | | |
| Signalment | | | |
| Public contact | | | |

Individual Elephant Risk Factors

- | | |
|--|--|
| Sex | Age |
| Species | Captive born |
| Wild caught – where, when | Dam reared or hand-raised |
| Spatial/Temporal | Herd size, social management, demographics and design |
| Reproductive history - number of calves, calf history | Geographic location of facility by regions |
| Facility design (space, airflow) | History of TB in facility – testing history and results |
| History of TB exposure (current and historical) | History of TB Testing |
| Treatment history for TB | Treatment history of other diseases |
| History of other significant medical events for individual elephant | Co-infections in herd (EEHV, Salmonella) |
| Caretaker history (foreign born, TB status, TB history) | Staff contact (duration, “close” contact intensity of contact) |
| Public contact (duration, intensity of contact) | Has management styles changed with the elephant? |
| Management style - Protected versus free contact, Management system (protected, free, mixed) | |
| History of movement of elephant, travel time, with whom, numbers, where | |

Development of a Reporting Form in order to do an epidemiologic study of risk factors for tuberculosis in elephants:
 Establish repository that is anonymous, private, non-profit, not “FOIAable”, that can link with the sample repository and with prospective studies (data with submissions). Potential partners are the Elephant Managers Association and the International Elephant Foundation. Need to develop a questionnaire of culture history, serologic history, isolate, TB monitoring data, staff conversion.

WORKING GROUP: Epidemiology/Data Management

| Goal/ Objective | Action Steps | Responsible Parties | Due Date |
|--|--|---------------------|-------------------------|
| Definitions | Create operational definitions for exposure, infection, disease. These will be part of the larger epidemiological study that is being started by a subset of this group which includes Michele Miller, Sharon Deem and Ramiro Isaza; input from other stakeholders will be solicited as the process progresses. | | Now and again with data |
| Standardized reporting/submission form | Develop a form that collects information regarding an individual elephant at the time samples for TB testing are submitted; identifying information to be coded by independent person. The standardized form is being developed as part of the diagnostics group. An informal proposal to use IEF to provide “codes” to facilities for their individual animals, similar to the herpesvirus program has been made and is being considered. | | October 1st |
| Establish repository | Identify data repository location Data repository may be different than sample repository (or same location). One proposal was to use the IMLS system for data – MM to investigate possibilities. Develop committee that will review request for use of samples Process of how this been currently AZA SSP steering committee reviews request for any AZA samples. Could use this system with IEF, EMA, non-AZA representatives? | | October 1st |
| Epidemiological data collection to evaluate risk factors (i.e. exposure) | Identify key risk factors using a survey of elephant stakeholders to prioritize data collection Exposed, becoming infected and progress to disease – need to define exposure, infection, and disease risks | This group | Today! |
| | Create a cross-sectional survey of elephant owners/facilities that will collect key data on risk factors, etc. on a standardized form to be part of epidemiological study Proposal to IEF has been submitted by members of this subgroup to start study. MM taking lead with input and support by other members. | | 12-24 months |
| | Collect current data from USDA testing that has been coded for retrospective study and pursue additional information on risk factors based on cross-sectional study results. Due to confidentiality, will only be able to get summary data from USDA-APHIS. Therefore, epidemiological study will need to include historical information on test results to address this point. | | |
| | Apply to IEF (or other funding) to fund prospective epidemiological study | | Done |

Occupational Risk Working Group

Kristy Bradley

Darryl Hoffman

Mike McClure

Janet Payeur

Joan Galvin

Ramiro Isaza

Rendi Murphree

The Occupational Health Working Group began their discussions with assessing participants' beliefs regarding the risk of transmission of *Mycobacterium tuberculosis* from infected elephants to humans. It was acknowledged that a relatively large proportion of persons whose work involves elephants (facility managers, animal handlers, communication specialists, etc.) are skeptical that zoonotic transmission of *M. tuberculosis* has been sufficiently documented. Some Working Group members expressed that there is more concern of transmission of TB from an infected worker to an elephant, and that relative to other occupational health risks associated with elephants, such as injury, the concerns of elephant-to-human transmission of *M. tuberculosis* are not that remarkable.

For the purpose of planning an epidemiologic study to quantify the occupational risk associated with TB and elephants, occupational risk will be assessed using employees who work directly with elephants. Occupational risk will be defined by the amount of time animal handlers spend with elephants and the elephants' TB status. Study subjects will be selected through membership in the Elephant Managers Association or other occupational affiliation. A subject's TB status will be determined based on testing using the QuantiFERON-TB test (or Interferon-gamma release assay), and a questionnaire will be administered to determine each subject's TB risk factors, type and duration of occupational activities related to elephants, and previous medical history. This will be a retrospective cohort study using only full-time elephant handlers based on a guesstimate that 900 people have worked with, or are currently working with elephants. Ideally, the selected cohort will include a randomized sample of 60 people potentially exposed to an elephant with active TB disease (i.e., at least one *M. tuberculosis* culture-positive elephant in herd with which the handler work(s)ed) and 60 people with no known TB exposure through elephant handling. Each subject would have a one-time blood sample taken that would be analyzed at a licensed human laboratory.

Members of the Occupational Health Working Group expressed and discussed some concerns regarding the proposed study. These concerns included potential selection bias due to a large degree of self-selection for participation in the study. There is a likelihood that institutional barriers placed on staff as to whether they can participate in the study may affect getting maximum participation and therefore a representative cohort for the study. There was also a concern regarding the study design of over-estimating the risk and that is being evaluated. It is important to note that this planned study focuses on personnel who work directly with elephants. However, in the two public health reports that were published in peer-reviewed journals that described human infection with *M. tuberculosis* through infected elephant herds, persons at-risk were more likely to involve employees at facilities who

had other job duties and responsibilities, e.g., groundskeeper, administrative, etc., and were exposed to *M. tuberculosis* by breathing aerosols in shared, indoor working environments.

| WORKING GROUP: Occupational Health | | | |
|---|--|----------------------------|---------------------|
| Goal/ Objective | Action Steps | Responsible Parties | Due Date |
| <p>There is a need to determine the risk of infection to elephant workers (defined as all inclusive from barn workers to veterinarians). Details of a planned occupational health study were discussed to determine how well the study may be able to answer this question.</p> | <p>Action Steps (Possible resources & partners)</p> <p>Study Design (Nat Assn of state public health veterinarians, CDC, USDA, academia, EMA, state vets, individual elephant holding facilities, etc.)</p> <p>Collaborative effort</p> <p>Retrospective study</p> <p>Subset of this group to further elucidate this project</p> <p>Ensure full participation and access to records/stakeholder buy in (EMA, AZA, IEF, etc.)</p> | Ramiro Isaza | February 1, 2012 |
| Find funding | This study has been funded | Ramiro Isaza | Funds are available |

Treatment Working Group

Dr. Lisa Armitige Susan Mikota
Dennis Schmitt Erica Lipanovich
Ralph Zimmerman Martha Weber
Genny Dumonceaux

Summary Priorities

- 1) Identify appropriate monitoring parameters
 - trunk washes, drug levels, temperature, weight, cbc, chemistry panel, electrophoresis.
- 1b) Develop standard protocol to collect samples on a standard schedule
 - trunk wash - series of 3 a week for two months followed by three in a week each month.
 - veterinarian to follow up on samples and request nucleic amplification testing (PCR) on first set or two of samples to facilitate needed information.
- 2) Better define adverse effect of treatment
 - develop a standardized daily monitoring form
- 3) Better define treatment protocol
 - adjust treatment based on current weights
 - this will be based on results of items 1, 1b and 2.
- 4) Identify or develop treatment biomarkers other than MAPIA
 - What are the most appropriate markers of treatment toxicity in elephants
- 5) Cost to benefit model
- 6) Formal retrospective study to drive future studies on data collection

Addressing priorities 3-6 will be predicated on the results of 1, 1b and 2.

WORKING GROUP: Treatment

| Goal/ Objective | Action Steps | Responsible Parties | Due Date |
|--|--|-------------------------------------|------------------|
| Gathering data is first step | Developing a data gathering form | Genny Dumonceaux & Erica Lipanovich | February 1, 2012 |
| Develop and disseminate standardization of appropriate monitoring parameters | <ul style="list-style-type: none"> • Trunk Wash Cultures – increase frequency for surveillance. Elephants on treatment - minimum of triple sample method weekly for the first two months and then decrease frequency to monthly monitoring; • Real-time PCR (commercially available and at State Lab – Microbacterial section); to reduce costs NVSL/state laboratories may batch samples; • Monitor animal’s weight; • Reevaluate drug dosages to correlate with weight; • Measure pharmacokinetic levels as often as possible; • Perform liver assays; • Measure Globulins; • Take elephant’s temperature; • Perform CBC, (hematocrit, total protein, and electrophoresis); and • Record behavior observations in journal of daily activities. | Genny Dumonceaux & Erica Lipanovich | February 1, 2012 |
| Standardize Sampling Protocols | <ul style="list-style-type: none"> • For standardization, identify or produce a trunk wash collection video. • Develop protocols to standardize the culture collection of other fluids; • Develop a universal sampling form | Susan Mikota | October 1, 2011 |
| Produce and disseminate data for drug monitoring | <ul style="list-style-type: none"> • INH – rectally start monitoring at time 0, 10, 30, 60 minutes (judgment may be necessary for earlier start time); • INH – orally start monitoring at time 30, 60, 120 minutes; • PZA – rectal/oral start monitoring 30, 60, 120, 180 minutes; • RIF – orally start monitoring 120, 180, 240 minutes; • EMB – orally 60, 90, 120 minutes; • Need fluoro pharmacokinetic studies for future recommendations • | Working Group | Ongoing |

| | | | |
|--|--|--|-----------------------------------|
| Develop and disseminate form to collect data on behavior and adverse effects to treatment. | Anorexia, weakness, diarrhea, red urine, red tears, weight loss, muscle tremors, leg/trunk paresis, red feces, photophobia, loss of stereotypic behaviors, lethargy, ataxia, in-coordination, joint pain, decreased water, Food type specific inappetance (produce and grain); attitude changes; insomnia; leaning | Included on the same form as that for recording drugs, dosing, sampling etc. | Done Appendix 1 |
| Better define and disseminate treatment protocols | Need to adjust treatment according to body weight. This will be based on the information on the above attained items. Highly predicated by the future data collected. | Working Group | Re-evaluate next Tb workshop 2012 |

Diagnostics and Immunology Working Group

Kay Backues Henry Boom
Paul Calle AJ Marlar
Joel Maslow Kari Johnson
Shannon Ferrell

With input from Deborah Olson, Tom Albert, Joan Galvin

Attendees identified the following 2 major concerns

1. Focus on PPV and NPPV of MAPIA and ElephantTB STAT-PAK® by entire stakeholders sharing data.
2. Promote, ID and facilitate new diagnostic tests for diagnosis of elephant TB with focus on antigen based tests.

The group developed a definition for **latency**. The same problem of defining latency exists in humans as there is little evidence to go on. In humans, an individual is considered latent if they have a positive serologic test, clear x-rays and no sign of disease.

For elephants, we need an Operational and Regulatory definition of latency

Operational – From a research point of view positive antibody test/ serologic test in an untreated animal followed for some period of time and not showing any signs of overt disease would be considered latent. Upon necropsy, granulomas are found in the lungs or lymph nodes.

Regulatory – At this time we only have serologic test available.

Upon necropsy, if tuberculosis is cultured, the elephant still could have been latent. The organism load would be so low there would not be a concern of transmission.

In a serological positive animal that is trunk wash negative it is unknown what the probability is that the animal is shedding. To answer that question the reliability of the trunk wash test needs to be determined. In a positive animal will serial trunk washes be consistently positive or intermittently positive, and what type of testing schedule will provide that answer?

The working group agreed that a regulatory definition of ElephantTB STAT-PAK® and MAPIA positive would be sero-reactive. Regulatory definition of ElephantTB STAT-PAK® positive is nonspecific reactor. An infected animal is trunk wash positive for both operational and regulatory purposes.

It is the opinion of this working group that based on our understanding today, the humoral based tests cannot distinguish between infected and showing clinical signs.

WORKING GROUP: Diagnostics & Immunology

| Goal/ Objective | Action Steps | Responsible Parties | Due Date |
|---|--|---------------------|--------------|
| Manage investigation of new diagnostic methods | Merge diagnosis working group into already existing group (IEF, AAZV or combo) ID persons or team to be diagnosis working group | Working Group | Ongoing |
| Identify TB and drug resistance- very promising Evaluate Genexpert | Select diagnostics to investigate, Genexpert, IGRA, biomarkers | Working group | 1 year |
| Developed a method to share data | <ul style="list-style-type: none"> • Create a committee, “Diagnostic Committee for Elephant Health”. This Committee will be comprised of a member of the following organizations: IEF, EMA, OABA, AAZV, and AZA. The executive committee of each organization will assign a member to serve on this committee. There will also be the ability to have an at large member who is an expert in MTB, preferably an infectious disease expert or immunologist from veterinary or human side as needed. The committee will decide when this is necessary and the selected person. • Committee will set up a standardized submission form – anonymous not traced back to any particular institution or management style with an anonymous numbering system for each participating institution and animal. • The set up and basic administrative duties may be located at the IEF office. • Survey membership institutions sample banks with a standardized sample and animal medical history form. • The sample bank will initially be electronic with potential in the future of making it a physical bank at some point in the future. <ol style="list-style-type: none"> a. Need expertise to set up database, standardized info and make columns status searchable. b. Assign status to each animal. c. Info on form, basic signalment, age, sex, species, amount of serum, time frame of serum (x # of years), status on ElephantTB STAT-PAK®, MAPIA and TW status and chronological data. Tissues in freezer, etc | Kay Backues | January 2012 |

| | | | |
|--|--|---------------|---------|
| Communication and Collaboration | Sample sharing and diagnostic working group to have website for collaboration. Apply for funding for diagnostics ID of interest, IMLS, MAF, AAZV, DAK | Working Group | |
| Define PPV/NPPV of MAPIA/ ElephantTB STAT-PAK® | Encourage data submission Michele Miller has retrospective data that will give us PPV/NPPV etc MOA of AAZV, IEF, EMA to contribute to these efforts. | Working Group | ongoing |

The Sample (tissue/serum) Bank will have the following Rules/Requirements:

- Committee will survey what samples are out there and establish sample categories
- Committee will standardize submission form
- Compile and have central site, website/clearing house for current research ongoing in elephants, to be focused on TB first. Potentially other studies may be able to take advantage of this resource.
- Researchers will submit a research proposal to be vetted by the committee
- Committee can identify research areas needed and potential researchers.
- Researchers agree that samples acquired and data generated cannot be shared with others for other purposes, studies, etc. Samples will be used for that specific research purpose only and any unused samples will be returned or destroyed
- Researchers agree to publish data generated in a peer reviewed scientific journal or if data is not suitable for peer review a white paper of the results – this will ensure accountability.
- Results must be reported to the committee as journal or non-peer reviewed document. This will avoid repeat of studies and prevent new diagnostics that don't appear to work
- Committee is tasked with reducing the waste of samples by minimizing the dissemination of samples
- Committee will promote pilot studies first to identify promising avenues and to conserve sample bank
- A contract will be developed so that each institution that participated would have assurance that they would be absolved of liability from sample use. For example, if someone can be injured by working with the sample, the institution who submitted the sample cannot be sued.
- Sample bank will not include isolates initially due to requirements for storage that are much more complicated – potentially down the road
- It would be good if a set of reference samples were identified so that a researcher could request a known + or only ElephantTB STAT-PAK® + samples. This will be set up in the data base by assigning each animal a status. For example:
 - ElephantTB STAT-PAK® + only = nonspecific reaction
 - ElephantTB STAT-PAK® and MAPIA + is a = seropositive reactor
 - ElephantTB STAT-PAK® +, MAPIA + TW+ = an infected animal



Conclusion

Conclusion

At the end of two days of discussions and break-out sessions, several areas of general consensus and/or proposed action emerged:

- The general public **is not** at significant risk of contracting MTB from elephants by attending zoos, fairs or circuses. This was confirmed time and again by infectious disease experts in the room.
- More research is needed on pathways of transmission of MTB from elephant to elephant as well as from humans to elephants and elephants to humans.
- To establish the risk of tuberculosis infection among elephant care givers, an epidemiologic study of the elephant handlers is needed. A retrospective study of the occupational health risks for elephant handlers working with MTB infected elephants is proposed, and implementation of the project is anticipated within the year.
- A better understanding of “exposure” is needed to differentiate high risk of transmission situations from those with little or no risk, and to assist in management decisions affecting the housing and management of elephants that are MTB infected, or exposed to MTB infected animals. To address this topic, epidemiological studies of all elephants in the U.S. are proposed. To avoid bias, a blind cross-sectional study (individual elephants would be assigned a number) would look at all potential risk factors (i.e. past medical history of the individual and herd, staff health, facility design, geographical location, etc). There would also be a retrospective study to obtain more detailed information. A subsequent prospective study could be developed to obtain further information.
- The use of the serological (blood) tests as accurate indicators of MTB infected elephants are still not completely accepted. Some believe these tests are inconclusive and should not at this time serve as the sole basis for travel restrictions or treatment, absent other indicators. Serological tests can be, however, useful tools in screening and to determine whether an animal should receive additional scrutiny and testing.
- There is a need to better understand and differentiate between elephants with latent versus active MTB infection. The definition of “latent” infection is also not completely agreed upon in the human tuberculosis community. Some cases of MTB have only been identified during post-mortem where small granulomas contained walled off MTB organisms; those cases might be considered latent, as the infection was contained and the elephant was not infectious at the time of death.
- A ‘serum and tissue bank’ was proposed to gather samples from the U.S. elephant community to serve as a resource for further diagnostic and research projects. This “serum and tissue bank” would be overseen by a group of stakeholders (i.e. AAZV, AZA, IEF, EMA, etc). This may include the current AZA serum and tissue bank.
- From these discussions several projects have been identified and will be developed further to improve the understanding of MTB in elephants (i.e. exposure, diagnostics, etc), and the possible risk factors to human staff.
- Developing standard forms for MTB sample submission, treatments, and data collection was discussed and is an actionable goal.
- Some members of the Treatment Working group proposed a shorter treatment duration (6 months instead of one year) with 1-2 drugs given every other day instead of every day, in order to reduce serious side effects which are suspected to be caused by treatment in

elephants using current protocols. This treatment regiment must be further studied and defined to ensure that the treatment is effective and it would not encourage the development of MDR strains.

- Although not unanimous, many participants believe that the 2008 Guidelines should remain in place while further epidemiological information is gathered, evaluated and analyzed. Some changes were proposed: 1) involving the state epidemiologist and/or state public health veterinarian; 2) Review how an animal can be removed from Group 3 through testing; 3) define meaning of ElephantTB STAT-PAK® reactive/non-reactive; and 4) clarify the process for handling elephants with various combinations of ElephantTB STAT-PAK®/MAPIA results. However, some participants supported adoption of the 2010 guidelines without delay.
- USDA-APHIS/AC has accepted but not yet implemented the 2010 Guidelines for the Control of Tuberculosis in Elephants. Although not unanimous, many of the meeting participants agreed to make a request that USDA continue to implement the 2008 Guidelines with some added clarifications. Many of the meeting participants also agreed to request that USDA not implement the 2010 Guidelines until changes can be made reflecting some of the recommendations made at this meeting, that the results of the initial ElephantTB STAT-PAK® and MAPIA testing be reviewed and interpreted, or until further studies are completed. However, some participants supported implementation of the 2010 guidelines without delay.

This report of the meeting including the recommendations and action items will be transmitted to Dr. Gipson and the USDA –APHIS/AC and to all stakeholder groups.

**Appendix 1
Sampling Form for Elephants
By Dr. Genny Dumonceaux**

Facility: _____ Date: _____

Identification: _____ Sex _____ DOB/Estimated Age _____ Weight _____ kg/lb

Time When Treated _____ Staff _____

Medications :

| Drugs | Dosage (mg/kg) | Frequency | Oral | Rectal | Retention Time | Form | Volume | Amount Received |
|--------------|----------------|-----------|------|--------|----------------|------------|--------|-----------------|
| Isoniazid | | | | | | C / W / CM | | |
| Rifampin | | | | | | C / W / CM | | |
| Ethambutol | | | | | | C / W / CM | | |
| Pyrazinamide | | | | | | C / W / CM | | |
| Levofloxacin | | | | | | C / W / CM | | |
| Moxifloxacin | | | | | | C / W / CM | | |
| Enrofloxacin | | | | | | C / W / CM | | |
| Pyridoxine | | | | | | C / W / CM | | |
| Amikacin | | | | | | Injectable | | |

C=Capsule, W=Water, CM=Compounding; Volume: Amount of Suspension

Sample collection time: _____ **Processing Time:** _____

Blood draw for drug level* : Drug tested _____ 0 min

post-5min post-15min post-1hr post-2hr post-4hr

Drug tested _____ 0 min

post-15min post-1hr post-2hr post-3hr post-5hr

Drug tested _____ 0 min

post-15min post-1hr post-2hr post-3hr post-5hr

*Adjust times as necessary.

Blood draw for other: CBC Chemistry Immune Bank RT/MAPIA Urine

Behaviors :

- Depressed
- Ataxia
- Lethargy
- Incoordination
- Lameness
- Insomnia
- Weak
- Aggitation
- Tremors
- Pain (muscle/joint)
- Paresis (leg/trunk)
- Other _____

Intake/Elimination:

- Anorexia
- Partial anorexia (concentrates/hay/produce)
- Red urine
- Red tears
- Loose stool
- Urination change
- No thirst
- Red feces
- Regurgitation
- Constipation
- Other _____

Comments: