

**Summary**  
**Elephant Tuberculosis Research Workshop**  
**Orlando, FL     May 21-22, 2005**

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Background: This document represents the summary findings of an Elephant Tuberculosis Research Workshop organized by Susan Mikota, DVM of Elephant Care International ([www.elephantcare.org](http://www.elephantcare.org)) and Michele Miller, DVM, PhD, the Veterinary Advisor to the AZA Elephant SSP/TAG.

The goals of this workshop were to 1) assess the status of experimental diagnostic tests; 2) evaluate experience gained from elephants that have been treated for TB; and 3) identify specific areas of research that will advance our knowledge of the diagnosis and treatment of TB in elephants.

Specific objectives:

- Review data pertaining to existing diagnostic tests
- Discuss experimental diagnostic tests and make recommendations regarding their validation
- Determine criteria for appropriate interpretation of experimental serologic data based on scientific principles
- Assess whether atypical mycobacterial infection(s) may impact diagnostic results
- Identify the goal of treatment in elephants – “cure” versus cessation of shedding
- Reevaluate current therapeutic regimens for elephants
- Investigate the effect of treatment on diagnostic test results
- Discuss means to improve our knowledge of the pathophysiology and epidemiology of TB in elephants

Participants included veterinarians with experience in the treatment of TB in elephants, regulatory representatives, mycobacterial researchers, and an M.D. with human TB expertise. A list of participants is included in Appendix I. All participants were given an opportunity to review and comment on this summary document.

Participants provided an update on their research or areas of expertise and were then divided into two working groups (Diagnostics and Therapeutics). Each working group was charged with identifying specific “next steps” needed to improve the diagnosis and treatment of elephant TB.

A synopsis of the information presented is included in this document followed by the summary recommendations of the Diagnostics Working Group and the Therapeutics Working Group.

Note: The current Guidelines for Control of Tuberculosis in Elephants (2003) remain in effect. The Guidelines specify the trunk wash culture as the recognized diagnostic test for elephants and strongly recommend the submission of samples for ancillary testing (Section 4 Ancillary Screening / Diagnostic Tests). At this time all samples for ancillary diagnostic testing should be submitted to Dr. Michele Miller. A submission form can be found at the following link:

[http://www.elephantcare.org/protodoc\\_files/new03/Elephant%20Serum%20Bank%20Submission%20Form.pdf](http://www.elephantcare.org/protodoc_files/new03/Elephant%20Serum%20Bank%20Submission%20Form.pdf)

Based on the findings of this Workshop and the collection of additional data, a subsequent meeting is planned for early 2006 to review specific recommendations for potential changes to the Guidelines.

Definitions: See Appendix II for abbreviations and definitions used in this report. Note that *Mycobacterium tuberculosis* and *Mycobacterium bovis* are collectively referred to as TB.

References: See Appendix IV for a bibliography of articles pertaining to elephant TB.

## **I. Brief History of Tuberculosis in Elephants**

Ancient treatises suggest that tuberculosis may have been detected as early as 2000 years ago in Asian elephants. The first zoo elephant reportedly affected by TB was an Indian elephant that died at the London Zoo in 1875. Additional cases appeared in the literature in the early 20<sup>th</sup> century. The first case of mycobacterial infection in an African elephant was reported in the 1960's.

Although additional cases were reported sporadically, tuberculosis "emerged" as a disease of concern for elephants in 1996 when two elephants from a privately-owned traveling herd died from the disease. This diagnosis in 1996 raised public concern for elephant and human health. In response the USDA formed an advisory panel to establish diagnostic and treatment protocols for the remaining elephants in the herd. The following year, five new elephant cases were identified. This prompted the development of protocols for the surveillance of all elephants. In 1998, the USDA and National Tuberculosis Working Group for Zoo and Wildlife Species released the first Guidelines for the Control of TB in Elephants, which were published as Policy 21 under the Animal Welfare Act. These guidelines established culture as the recommended test for the diagnosis of TB in elephants, and classified elephants into groups based on culture results and exposure history.

During this time, treatment regimens were being developed and tested. Based on changes in the knowledge base of TB in elephants, the Guidelines were revised in 2000 and again in 2003. Major changes in the last revision included the requirement that drugs be directly administered, rather than given free choice in food. Target serum drug levels were established and treatment regimens and options were updated.

Between 1994 and June 2005, there were 34 confirmed cases of tuberculosis in elephants in the U.S. population. Thirty one Asian and three African elephants were affected. *Mycobacterium tuberculosis* was the etiologic agent in 33 cases and *M. bovis* in one case. Three cases of tuberculosis caused by unusual non-tuberculous mycobacteria are excluded from this discussion. Twenty-two of the 34 cases were diagnosed antemortem on the basis of cultures obtained by trunk washes or swabs and 12 cases were diagnosed postmortem. The majority of elephants, including the 12 cases diagnosed at post-mortem, did not show clinical signs suggestive of TB. Of the 34 cases, 19 have died or were euthanized. In some cases TB was an incidental finding at necropsy and was not considered the cause of death.

The zoonotic potential for the spread of TB between elephants and humans has been documented in one case (Michalak et al. 1998). Other reports have demonstrated epizootic spread of the same strain of *M. tuberculosis* between elephants and other zoo mammals (Oh, et al. 2002).

Currently, the diagnosis of TB in elephants remains a dilemma. The sensitivity of trunk wash culture, the currently recommended test for diagnosis, is unknown. False negatives have been documented (trunk wash negative elephants that were subsequently found to be culture positive at necropsy). Although unlikely, false positive results may also occur due to incorrect labeling of specimens at the time of collection; cross contamination of specimens has been documented using older microbiologic growth systems but appears to be decidedly rare at present.

Other non-culture techniques for TB diagnosis include ELISA, PCR, gamma interferon (under development), and lymphocyte stimulation (not available at this time). Serologic or other indirect assays (that detect antibodies but not the actual organism) may not be able to differentiate among 1) animals that are infected and shedding 2) animals that are infected and not shedding and 3) non-infected animals that were previously infected. Potential future diagnostic tests might utilize those being developed for human patients.

Similar to diagnostic issues, treatment parameters are still under investigation. Effective drug levels for elephants have not been determined however, levels known to be effective in humans have been achieved in elephants. Adverse drug effects have been a significant issue for some elephants. Long-term medication administration is challenging in this species, and there is currently no pre-mortem method to confirm a cure. However, there are a number of new anti-tuberculosis drugs being developed for humans that may be more effective or have fewer side effects. Other routes and treatment regimens (route and frequency) could also be explored for future treatment options.

## **II. Diagnostic Testing for Tuberculosis in Elephants**

### **A. Culture and MTD (nucleic acid amplification test)**

A summary of culture results shows that TB has been isolated from 34 captive elephants in 15 herds in 8 different states between 1994-June 2005 (includes one case of *M. bovis*). In three cases, *M. tb* recurred following initial treatment. Isolates from 12 elephants showed resistance to some anti-TB drugs.

Trunk wash samples may be submitted to any microbiologic laboratory capable of culturing for mycobacteria. Over the period 1997-2005, 8715 trunk washes or swabs were submitted to National Veterinary Services Laboratories (NVSL) that yielded 423 different mycobacterial isolations. *Mycobacterium avium*, a non-tuberculous mycobacterium, was the most prevalent mycobacterial species detected (Table 1). As with humans, infection with non-tuberculous mycobacteria (NTM) is not considered to represent a public health risk or a risk to other animals. Moreover, there is no data to suggest that NTM are communicable, i.e. spread from animal to animal or animal to human.

DNA fingerprinting (RFLP) of some of the elephant isolates has identified 7 different strains of *M. tuberculosis*. These were found in 6 different areas of the country. Animals from the same facilities often had identical or similar RFLP

profiles, which suggested transmission of infection among the elephants or a common source of infection. However, one facility had 4 elephants with 3 unique strains of *M. tb*. In one facility, two out of five culture-positive elephants were found to have two different strains of *M. tb* each.

NVSL has also performed over 1100 Gen Probe MTDs (a nucleic acid amplification test). The Gen Probe MTD is approved by the FDA for the diagnosis of TB from human sputum and bronchial specimens and tracheal aspirates; the test does not differentiate between the presence of *M. tuberculosis* or *M. bovis*. A total of 72 submitted specimens were MTD positive. Of the 72 positive samples, *M. tb* was isolated by culture from 13 samples; 28 samples had suspect cultures (6 with *Mycobacterium sp.* not otherwise identified). There were 1000 MTD negative samples from which *M. tb* was cultured from 6 samples. Therefore, this test identified 13 culture positive elephants correctly. It also correctly identified the *M. bovis* elephant. However, there were MTD positive elephant samples from which *M. tb* was not isolated by culture and six MTD negative elephants had a positive *M. tb* culture.

Of the 34 culture positive elephants, 22 have been treated. Three of the 22 were treated twice. One of the re-treated elephants was an early case in which anti-TB drugs were given ad lib over food, a method that is no longer considered adequate. In a survey of antibiotic resistance of 19 elephants at 10 facilities that included 34 isolates of *M. tb* and 1 isolate of *M. bovis*, it was found that 9/34 strains (26.5%) were resistant to at least one antibiotic (Harris and Osorio, NVSL) (Table 2). Human TB drug resistance is reported to be approximately 10%. Similar to humans, elephant isolates were most commonly resistant to isoniazid and rifampin. Multi-drug resistance (defined as resistance to two or more first line anti-TB drugs) was observed in two elephant isolates at NVSL.

In summary, the RFLP profiles showed that multiple strains of *M. tuberculosis* were recovered from geographically unrelated elephants. This suggests that there were several sources of infection rather than a single index case spreading to multiple elephants. The emergence of drug-resistant strains may be associated with inadequate antibiotic treatment.

### **B. ELISA (Enzyme-linked immunosorbent assay)**

Earlier versions of this assay incorporated multiple antigens, including a six antigen panel from *M. bovis* (CF, PPD, MPB 70), *M. tuberculosis* (ERD, RA), and *M. avium* (AVPPD). Except for MPB 70, all represent antigen pools of culture filtrate (CF), purified protein from the culture filtrate (PPD-bovis), or protein mixtures from pathogenic human *M. tuberculosis* strains Erdman and H37Ra. Sera from Asian elephants that were culture positive for *M. tuberculosis* by trunk wash were used as positive samples. No treatment was administered prior to sampling (n=7). Negative samples were obtained from 25 Asian and 15 African elephants. Criteria were a negative trunk wash culture within 3 months of serum sample, no contact with known TB positive elephants, and no movement outside the holding institution within the last 5 years. Also, no intradermal tuberculin test had been administered to either group within 6 months prior to sampling (Larsen et al. 2000).

Differences in seroreactivity were observed between infected and non-infected elephants for all antigens except MPB70. In addition, there were differences in seroreactivity in non-infected Asian and African elephant samples for CF, PPD, ERD, and AVPPD. Using CF + RA in the ELISA, the specificity and sensitivity of the test were 100% for detecting culture positive elephants. (See Appendix II for definitions of sensitivity and specificity). When a small number of elephants had intradermal skin tests, it appeared that some had a change in seroreactivity. There also appeared to be a change in seroreactivity with treatment in some elephants.

A modified ELISA using six antigens has been recently tested (*M bovis* – CF, MPB70; *M tb* - ESAT6, Ag85, MPT64, MTP32). Using this assay a group of 25 Asian elephants were tested, 6 culture positive and 19 culture negative, based on the criteria above. Antigens that appeared to discriminate between culture positive and negative samples were CF, MPB70 and ESAT6. An expanded study was performed with 68 Asian elephants, including 16 culture positive animals. Using CF + ESAT6, the specificity was 100% and sensitivity was 94% (one culture positive elephant showed no seroreactivity). Therefore, this ELISA correctly classified 67 out of 68 elephants tested. ELISA was capable of differentiating infected elephants earlier than culture (months-years).

### **C. PCR (polymerase chain reaction)**

Studies are underway to examine the ability to detect mycobacterial organisms in trunk wash samples using PCR. Currently, experiments have been performed using *M. bovis* spiked trunk wash. The test method has detected concentration of 100 organisms/100 ul of sample. Additional work needs to be performed to determine sensitivity of the assay and run PCR on trunk washes from known TB infected elephants.

### **D. Rapid Test, MAPIA (MultiAntigen Print ImmunoAssay), and Immunoblot**

One company, Chembio, has developed two tests for the diagnosis of TB in animals. The Rapid test (RT) is an immunoassay based on lateral-flow technology. Nitrocellulose membranes are impregnated with test antigens in a single test strip as a screening test with relatively high sensitivity but lower specificity. The MAPIA assesses the presence of antibodies to ~10 individual mycobacterial antigens. The RT and MAPIA are developed as screening and confirmatory tests similar to current technologies in use for HIV testing.

The RT uses a mixed grouping of selected mycobacterial antigens using an automatic printing device. Strips are laminated with sample pad, conjugate pad and sink pad, and placed into plastic cassettes similar to a pregnancy test kit. The test employs a blue latex-based antibody detection system using antigen-coupled particles. Thirty microliters of elephant serum, plasma, or fresh whole blood is placed onto the sample pad, and three drops of diluent are added. Test results can be read within 20 minutes. A blue band of any intensity observed in the test window is considered a positive result while no visible band in the test window is a negative result. A control line developed in the control window will appear irrespective of the presence of specific antibody in the sample to confirm that the test is performed properly (Figure 1).

The MAPIA uses 10 purified recombinant antigens of *M. tuberculosis* (ESAT-6, CFP10, MPB59, MPB64, MPB70, MPB83, alpha-crystallin, 38 kDa protein, Mtb8, Mtb48), 3 polyprotein fusions (TBF10, CFP10/ESAT-6, and Acr1/MPB83), and culture filtrates of *M. bovis* that are separately immobilized on a nitrocellulose membrane using a semi-automated airbrush-printing device. The membrane is cut into strips, which are incubated with elephant serum samples. After incubation with elephant serum (or whole blood), antibodies bound to printed antigens are visualized using a species-nonspecific conjugate (Figure 2). Antigen for the immunoblot is a whole cell sonicate (WCS) of *M. bovis* that is separated on polyacrylamide gel and transferred to nitrocellulose membranes. Similar to MAPIA, these membranes are incubated with elephant serum then visualized using a detection reagent.

Multiple serum samples collected from 90 Asian and African elephants in Europe, South Africa, and the U.S. have been tested using the MAPIA and RT. Of these, 17 were culture-positive for *M. tuberculosis* (or *M. bovis* in one African elephant) (Table 3). Of 63 culture negative control elephants with finalized status (healthy or other disease), one was reactive with RT but not MAPIA (this animal had a chronic osteomyelitis). The antibody responses in culture positive elephants to individual antigens demonstrated that the immunodominant antigens were ESAT-6 (100% sensitivity) and CPF10 (75%). Overall, with this test population, the Rapid Test showed 100% sensitivity and 98% specificity; the MAPIA had 94% sensitivity and 100% specificity.

Using the Rapid Test, MAPIA, and immunoblot, sera were tested from known culture positive elephants over time to examine the development of the serologic response. Individual antibody responses showed that all three assays could detect seroconversion years prior to the first positive culture in a number of infected elephants. In one epidemiological study, it was demonstrated that in a group of 5 infected elephants (confirmed at necropsy), there were only 6 positive cultures out of 59 trunk wash samples. One elephant never had a positive trunk wash culture and was diagnosed at necropsy. However, retrospective evaluation of serum from this elephant showed positive results on the Rapid Test-as long as 8 years prior to euthanasia.

These assays were also used to test sera from culture positive elephants before, during, and after treatment for tuberculosis. The pattern of antigen seroreactivity in MAPIA and immunoblot changed as treatment was initiated, and in those elephants with recrudescence, a rise in titer to specific antigens was also detected.

Serum from two elephants infected with *Mycobacterium szulgai*, were positive on RT, but had distinctive MAPIA patterns from those observed with *M. tb* culture positive elephants.

These serological studies produced two important observations. First, with all elephant TB cases where retrospective samples were available for testing, the antibody responses could be detected much earlier (2 to 6 years) than positive cultures from trunk washes were first documented. Moreover, in some instances, culture was positive only at necropsy (consistently negative from trunk washes) while serology indicated TB infection occurred years earlier. Second, when TB-infected elephants were under treatment, the antibody titers to certain antigens used

in MAPIA declined quickly to baseline levels, apparently in response to therapy. Thus, MAPIA may potentially be used for early diagnosis, and also for monitoring response to treatment. This was also shown in some cases with ELISA as well.

Further research is needed to validate these tests and determine the sensitivity and specificity (the true rate of false positive and false negative test results) using the RT and MAPIA. In particular, these tests have not been sufficiently evaluated in herds with a low pre-test probability of a positive test (where false positives are more likely). For animals with recent exposures, the time to develop a positive RT or MAPIA test result (i.e., the time to seroconversion) is unknown. Additionally, the lower level of detection of latent infection is unknown. For example, will the RT and MAPIA detect disease that is isolated to a single lymph node?

### **E. Other Diagnostic Tests**

At this time, other diagnostic tests have not been tested or are not currently available for use in elephants. A gamma interferon assay (that measures induction of gamma interferon by peripheral blood mononuclear cells of tested animals) is being developed for use in elephants, but it is not expected to be available for at least another few years. The BTB assay (a combination of lymphocyte stimulation and seroreactivity against bovine PPD) is no longer available.

### **Overview of Diagnostic Tests**

The ELISA, Rapid Test, MAPIA, and immunoblot detect antibodies in elephant sera to mycobacterial antigens; therefore, they provide indirect evidence of infection or exposure. Data presented suggest that these assays may be used to detect seropositive elephants months to years before a trunk wash or necropsy culture is positive. Patterns of seroreactivity may also be useful for monitoring response to treatment. Since the RT detects seroreactivity to all target antigens simultaneously and therefore cannot distinguish the pattern of seroreactivity, it would best be used as a screening test, with the MAPIA and/or ELISA as a confirmatory test. PCR of trunk wash samples has not been tested with field samples, but may provide another method of organism detection. Recent work by Sloutsky et al (2004) has determined that dilution of clinical specimens 1:10 increases the sensitivity of the MTD in human samples. Whether similar increases in test performance can be realized for elephant samples is unknown. Definitive diagnosis of infection still requires identification of the mycobacterial organism, usually by culture. Culture is essential to determine antibiotic sensitivity patterns and the selection of drugs that will be used for treatment.

**STATUS:** Culture of trunk wash samples is the only test currently required by the Guidelines for the Control of Tuberculosis in Elephants 2003. All other tests discussed in this document are considered ancillary tests at this time and further work is needed for validation.

### **III. Therapeutic Issues for Tuberculosis in Elephants**

#### **A. Current Anti-Tuberculosis Therapy, and Adverse Effects**

Commonly used drugs for treatment of tuberculosis in elephants include isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB). Consult the Guidelines for current recommended dosages and routes of administration. Initial empirical therapy has usually included INH, RIF and PZA. Drug resistance has been identified to INH and RIF. This may necessitate treatment regimens with limited choices of remaining first line agents (PZA, EMB), inclusion of second line agents) or the use of drugs for which there is significantly less clinical data (e.g. enrofloxacin or other quinolone antibiotics). See below for more details regarding treatment regimens.

Multiple clinicians have experienced similar problems in the treatment of TB in elephants. Elephants can be discerning eaters and difficult to treat orally, especially long-term. Direct oral administration appears to achieve slightly higher blood levels than the rectal route, and oral administration over food fed ad lib is no longer recommended. However, elephants can be trained to accept a bite block for oral administration with an equine dosing syringe. Some drugs, such as amikacin, can only be given by injection. In one case, this drug was formulated at a higher concentration to decrease the volume injected intramuscularly.

Other issues are cost and availability of drugs. It was estimated that the cost of treating a single elephant was \$50-60,000. Drug costs based on the recommended dosages for an adult Asian elephant were roughly \$5/day for INH, \$65/day for RIF, \$190/day for PZA, and \$155/day for EMB. Since therapy requires multiple drugs to be given, the costs can vary but are substantial. The other issue is the quantity and availability of drugs. Often bulk drug is used and obtaining this in adequate quantities may cause interruption in treatment.

Signs of toxicity in treated elephants were commonly seen and usually associated with INH. These included weakness, ataxia, anorexia, elevated liver enzymes, pica, and sand consumption. Clinical signs usually resolved with a reduction in dosage, stopping medication for a period of time, or using "pulse" therapy (ex. treating three times a week at double the dosage). It was often difficult to reach therapeutic blood levels without inducing toxicity, and there appeared to be individual variation between and within elephants on the same treatment regimen.

Since elephants are usually treated with multi-drug therapy for tuberculosis or prophylaxis, it is often difficult to determine which specific drug may be responsible for the clinical signs associated with toxicity. In one group of elephants treated prophylactically with enrofloxacin at 10 mg/kg PO SID and PZA at 30 mg/kg PO SID, clinical signs of inappetance, increased epiphora, and blepharitis were seen in the majority of individuals, with corneal edema and limb stiffness observed in half the group. One individual treated with PZA, EMB, enrofloxacin and amikacin developed anorexia, weakness, ataxia, and trunk paralysis/paresis. Another individual treated with INH and PZA developed anemia (decreased hematocrit, RBC count, and hemoglobin concentration), which resolved about 4 weeks after reduction in dosages of both drugs. Discussion with a pharmacist during this period indicated that INH and PZA both affect bone marrow RBC stem cells but through different pathways.

Complete blood count (CBC) and serum chemistry panels are recommended monitoring tools for elephants receiving anti-tuberculosis drugs. Elevated hepatic enzymes were commonly associated with clinical signs. Common human side effects of anti-tuberculosis drugs are shown in Figure 3.

### **B. Clinical Experiences with Tuberculosis and Effectiveness of Treatment in Elephants**

Similar to the information presented in the diagnostic section, veterinarians that have worked with TB-infected elephants have found culture to be a specific but insensitive method of diagnosing tuberculosis in elephants. Some elephants that had pulmonary pathology and from which mycobacteria were isolated at necropsy had histories of multiple negative trunk washes. Other elephants had variable numbers of negative trunk wash cultures (up to 54) before having a positive isolation. In one elephant with uterine mycobacteriosis, culture of vaginal discharge still only yielded positive isolation in 28.6% of the samples, with lower recovery from other sites.

Approximately 22 culture positive elephants have received anti-tuberculosis treatment to date. Based on serial cultures, elephants, like humans, appear to cease shedding within weeks of the initiation of treatment. Can TB be cured in elephants? Three elephants that were treated for TB and subsequently died or were euthanized were found to be culture negative at postmortem examination. Two other treated elephants were culture positive at necropsy. One of these was the 1996 index case. This elephant was not considered to have been adequately treated. The initial treatment was given with medications administered over food (prior to the change in the Guidelines) and variable drug levels were achieved. The elephant was treated a second time but again, treatment was not considered adequate and may not have followed prescribed Guidelines. The other case was an elephant that was under treatment for multi-drug resistant TB. This elephant was euthanized. Since only a few of the treated animals have died or been euthanized, it may be difficult to determine whether treatment has eliminated infectious organisms or just suppressed shedding.

### **C. New Compounds in the Anti-TB Arsenal**

The current first line agents for tuberculosis treatment in humans are isonicotinic acid hydrazide (INH), rifampin (RIF), pyrazinamide (PZA), ethambutol (EMB), and streptomycin (SM). Second line agents are capreomycin, amikacin, ethionamide (ETH), para-aminosalicylic acid (PAS), and the fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin). Multi-drug resistant (MDR) TB occurs in approximately 5-10% of cases worldwide, with the highest incidence in Russia, China, Mexico, Vietnam, and Haiti. It is most common among low socioeconomic groups and patients with poor treatment adherence. INH or RIF resistance is more prevalent at approximately 20%.

Human empiric treatment regimens for TB are determined by the prevalence of TB in the local population. In areas where MDR-TB is less than 1%, a three drug treatment using INH, RIF, and PZA is used. If the prevalence of MDR-TB is 1-5%, a four drug cocktail is used with INH, RIF, PZA, and EMB. When the prevalence of

MDR-TB is greater than 5%, a fifth drug is added to the above combination, usually streptomycin or a fluoroquinolone. When the susceptibility of a human TB isolate has been determined, treatment length and drug combination is based on this information (Figure 4).

Newer agents include the fluoroquinolones, such as gatifloxacin, levofloxacin, moxifloxacin, ciprofloxacin, and ofloxacin. These drugs have synergistic activity with INH and ethionamide. In clinical trials of small numbers of patients those for whom, moxifloxacin was added to their anti-tuberculous regimen sterilized quicker and had fewer relapses with less hepatitis than standard treatments.

Linezolid is a newer drug that is a combination of quinopristine and dalphinopristine. It is synergistic with rifampin and has been used to treat MDR cases. Although the MIC is <1mcg/ml, it is less commonly used due to toxicities such as thrombocytopenia and other cytopenias; other adverse effects such as hearing loss and visual disturbances have also been reported rarely.

There are a number of other natural and synthetic compounds developed but only a few have been tested in mice and one in humans. Diarylquinoline (R207910) is a quinolone derivative-like drug that has been well tolerated by mice and humans. It is bactericidal against MDR-TB including quinolone-resistant strains. However, it is not yet commercially available. Other TB active compounds include phenothiazines, 9-benzyl-6-(2-furyl) purines, capuramycin analogs, and biphenyl analogues of thiolactomycin as well as a variety of plant sources, such as *Piper sanctum*, *Juniperus procera*, *Euclea natalensis* root bark, Indian sponge, *Limnophila geoffrayi*, and *Senna lobliqua* stem and fruits.

### **Overview of Treatment Issues**

There have been 5 elephants treated for tuberculosis that have subsequently died or been euthanized. Three were culture negative at necropsy and two that had recrudescence disease, were positive. Of the two elephants that were culture positive at necropsy, one may not have received adequate treatment in compliance with protocols outlined in the Guidelines and the second was euthanized prior to completing treatment. Clinical signs associated with toxicity are common and complicate ability to follow treatment guidelines. Knowledge of transmissibility to other elephants and zoonotic potential is also limited; therefore, elephants in contact with infected animals are often treated prophylactically and may exhibit drug side effects. Drug resistant *M. tb* has been isolated from elephants and requires treatment with multiple first and second line drugs. New anti-TB drugs may provide safer more effective choices for treatment in the future.

**STATUS:** At the current time, combination drug therapy is recommended for treatment of tuberculosis in elephants as recommended in the Guidelines (2003). Preliminary data indicates that effective treatment may rapidly cease shedding and even eliminate culture positive organisms from lesions tested at necropsy (n=3). However, lack of early diagnosis, toxicity of drugs, and prevalence of MDR TB may complicate the ability to achieve a cure.

## IV. Workshop Recommendations

Based on the information presented, the current knowledge and future direction of diagnostic testing and treatment of tuberculosis in elephants was discussed. Concerns for the public health and regulatory consequences were taken into consideration. The past approximately 10 years of work in this field has yielded significant progress but there remain many gaps in knowledge regarding the pathobiology of the disease in elephants. In order to address some of the most immediate practical issues facing diagnosis and treatment, the following recommendations and action plans were developed.

### A. Diagnostics Working Group Recommendations

**In order to coordinate sample and data collection, it is recommended that serum samples continue to be sent through the Elephant SSP/TAG Serum Bank, to create a reference bank and provide sample availability to all researchers for test validation and data analyses. In addition, all elephants that die or are euthanized should receive a thorough necropsy and mycobacterial culture per SSP recommendations (see necropsy protocol at [www.elephantcare.org](http://www.elephantcare.org)).**

1. Correlate retrospective and future necropsy data with diagnostic test results and clinical history (culture, serologic tests, PCR, gross lesions, histopathology, and presence of acid-fast bacilli).
  - a. Request sera from previous cases for testing.
  - b. Obtain copies of necropsy reports and other lab data as needed.
  - c. Recommend thorough necropsy on ALL elephants with data collected as outlined above. Reinforce the existing recommendation of the Elephant SSP and the Guidelines that all elephants that die undergo a thorough necropsy.
  - d. Formation of necropsy teams to facilitate process.
2. Collate data collected through designated individual/group.
  - a. See statement above regarding Elephant SSP/TAG Serum Bank.
3. Perform data validation and comparison for currently available assays (ELISA, RT, MAPIA)
  - a. Will need to segregate data for statistical analyses (*M. bovis* vs *M. tb* vs other mycobacteria; African vs Asian elephants).
  - b. Calculate sensitivity, specificity, positive and negative predictive values of each test (and possibly tests in series).
4. Ensure that all participating research labs have access to same serum sample set.
5. Create a set of reference sera (“positive” and “negative”) that can be used to validate future assays.
  - a. Ideally, “negative” samples would be from necropsy negative animals (culture and histopathology). However, for these purposes, serum from any elephant found negative in all 3 serologic tests and culture negative, with no contact with a known TB elephant for 5 years, would be considered negative.

6. Recommend that all elephants have ante-mortem annual trunk wash cultures (triple samples) and serum be collected at the same time as trunk wash for ELISA/RT/MAPIA and for serum banking.
  - a. Current Guidelines already strongly recommend serum collection for ancillary testing; this is not a mandated change.
  - b. Recommend that any mycobacterial isolate from trunk wash or other site cultures that are not performed at NVSL, be sent to NVSL as a repository for future studies (epidemiology, etc.).
7. Perform retrospective epidemiological review of culture positive elephants using validated serological or antigen tests.
8. Recommend further studies of the pathobiology of mycobacterial infections including, *M. szulgai* and other non-TB mycobacterial infections in elephants
  - a. Retrospective review of cases.
  - b. Obtain sera from previous cases for serologic testing.
  - c. Review list of elephants that have had atypical mycobacteria (MOTT) isolated from trunk washes.
  - d. Obtain sera for serologic testing, if possible, to study the impact of exposure/infection on serologic results.
  - e. Review case history for possible correlation with clinical signs or pathology.
9. Request speciation of ALL mycobacteria isolated from elephants.
10. Recommend frequent serum collection of treated elephants for serologic monitoring (at least every 6 months, or more frequently).

## **B. Therapeutics Working Group Recommendations**

1. Investigate new routes of treatment, such as transdermal, inhalation.
2. Review current TB drug therapy in elephants.
  - a. Review current treatment regimens and consider individualizing drug levels by determining peaks at 1,2,4,6 hours for each drug (The current Guidelines specify serum drug levels be tested at 2 hours).
  - b. Collate successful treatment regimens (such as pulse therapy) for reference for others.
3. Create information sheet regarding clinicopathologic changes associated with drug toxicities.
  - a. Provide information regarding drug adverse effects, what to monitor, how to treat, and what drugs can be substituted or how treatment regimen can be changed.
  - b. Toxicity trial of PZA.
4. Investigate new TB drug options for elephants.
5. Pilot pharmacokinetic studies.
  - a. Compare commercial ethambutol to bulk product in terms of rectal irritation.
  - b. Pilot pharmacokinetic study of rectal rifampin.
  - c. Pilot pharmacokinetic study of amikacin at TB dose IM.

- d. Pilot a transdermal pharmacokinetic study with amikacin or other drugs that have limited routes of administration (ETH, RIF).
- 6. Recommend using serologic tests (ELISA, +/-RT, MAPIA) to monitor treated elephants every 3-6 months, along with culture.
  - a. Create a database of treated elephants to include date of first positive culture, treatment dates and regimens, adverse effects, drug resistance, recurrence, etc.

**Table 1. Number of elephant samples with mycobacterial isolates 1994-2005**

Mycobact. species	# samples	Mycobact. species	# samples
M. species <sup>a</sup>	114	M. gastri	2
M. avium	142	M. chitae	3
M. tuberculosis	35	M. scrofulaceum	3
M. terrae complex	9	M. phlei	2
M. gordonae	6	M. xenopi	2
M. fortuitum	7	M. szulgai	3
M. tb complex	2	M. bovis	1
M. ulcerans	2	RG IV	5
M. smegmatis	2	M. interjectum	2

(J. Payeur, NVSL)

<sup>a</sup>Early culture samples and some isolates were unable to be speciated.

**Table 2. Antibiotic susceptibility results for 34 samples (from 19 elephants)**

Antibiotic	Resistant isolates
Streptomycin	0/34 (0%)
Isoniazid	8/34 (23.5%)
Rifampin	2/34 (5.9%)
Ethambutol	0/34 (0%)
PZA	2/34* (5.9%) (*one <i>M. bovis</i> )

(J. Payeur, NVSL)

\* *M. bovis* is inherently PZA resistant.

**Table 3. Serodiagnosis of tuberculosis (*M. tb*, *M. bovis*) in elephants using Rapid Test (RT) and MAPIA**

Culture status	# elephants	# positive RT	# positive MAPIA
Culture negative	63	1	0
Culture positive	17	17	16

(K. Lyashchenko, Chembio, Inc.)

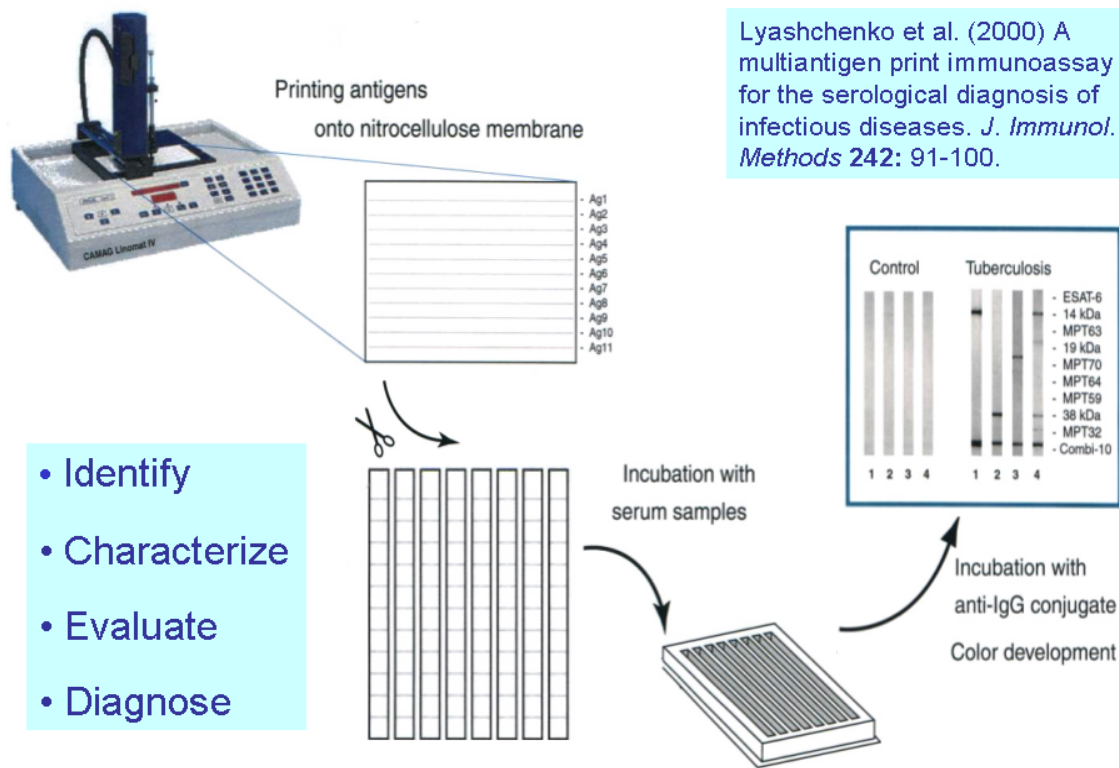
Figure 1. Rapid Test



(K. Lyashchenko, Chembio, Inc.)

Figure 2.

## MultiAntigen Print ImmunoAssay (MAPIA)



Lyashchenko et al. (2000) A multiantigen print immunoassay for the serological diagnosis of infectious diseases. *J. Immunol. Methods* 242: 91-100.

(K. Lyashchenko, Chembio, Inc.)

**Figure 3. Common human side effects of anti-tuberculosis drugs.**

**Rifampin human side effects**

- Anorexia, nausea, vomiting .....
- Thrombocytopenia
- Muscle weakness, limb pain, headaches, ataxia
- Visual disturbances
- Elevated BUN, serum uric acid
- Pruritis, urticaria, rash, conjunctivitis

**PZA human side effects**

- Hyperuricemia → gouty arthritis
- Hepatocellular damage
- Nausea, vomiting, anorexia
- Arthralgia, myalgia (frequent)
- Photosensitivity, porphyria, fever (rare)

**Isoniazid human side effects**

- Peripheral neuropathy
- Nausea, vomiting, epigastric distress
- Elevated liver enzymes, hepatitis
- Agranulocytosis (also in camels, ele)
- Skin eruptions, fever, vasculitis
- Pyridoxine deficiency
- Rheumatic syndrome, SLE like syndrome

**Quinolones human side effects**

- Nervousness, agitation, insomnia, anxiety, dizziness, convulsions(people)
- Photosensitivity
- Dermal rash or allergic reaction.
- Crystalluria (with alkaline urine)

**Ethambutol human side effects**

- Optic neuritis
- Acute renal failure

**Ethionamide human side effects**

- Nausea, vomiting, abd. Pain, diarrhea, hypersalivation, stomatitis, anorexia, wt loss
- Psychotic disturbance, depression, drowsiness, dizziness, restlessness, headache, postural hypotension.
- Increase serum bilirubin, SGOT, SGPT, hepatitis.
- Rash, photosensitivity, thrombocytopenia

(G. Dumonceaux, BGT)

**Figure 4. Human TB treatment regimen**

**Human TB Definitive Treatment**

- Fully susceptible strain
  - 2 months : INH / RIF / PZA
  - 4 months INH / RIF
- INH resistant
  - 2 months: RIF / EMB / PZA
  - 4 months: RIF / EMB or PZA
- MDR-TB (INH & RIF resistant)
  - 2 months EMB / PZA / FQ / SM or PAS or ETH
  - 10 months EMB / PZA / FQ (some use 4 drugs)

\* drugs and length of Rx based on susceptibilities

(J. Maslow)

## Appendix I - Workshop Participants

1. Wilbur Amand, VMD - Executive Director, American Association of Zoo Veterinarians
2. Ray Ball, DVM - Veterinarian, Busch Gardens-Tampa
3. Genevieve Dumonceaux, DVM - Veterinarian, Busch Gardens-Tampa
4. Freeland Dunker, DVM - Veterinarian, San Francisco Zoo
5. Karin Hamilton, Tufts veterinary student with the Nepal Elephant Project
6. Ramiro Isaza, DVM, MS, DACZM – Assistant Professor , University of Florida, College of Veterinary Medicine
7. Gretchen Kaufman, DVM – Assistant Professor, Tufts Center for Conservation Medicine; Nepal Elephant Project
8. Scott Larsen, DVM, MS, DACZM – Lecturer, University of California-Davis, School of Veterinary Medicine
9. John Lehnhardt – General Curator Disney’s Animal Kingdom; Elephant SSP/TAG Steering Committee
10. Bill Lindsay, DVM, DACVS – Veterinarian, Feld Entertainment Inc.
11. Konstantin Lyashchenko, PhD – Research Director of Mycobacterial Immunology, Chembio Diagnostic Systems, Inc.
12. Joel Maslow, MD, PhD – Chief of Infectious Diseases VA Medical Center, Associate Vice Dean for Research, Associate Professor of Medicine in Infectious Diseases, University of Pennsylvania
13. Susan Mikota, DVM – Director of Veterinary Programs and Research, Elephant Care International
14. Michele Miller, DVM, PhD – Veterinarian, Disney’s Animal Programs; Veterinary Advisor to the Elephant SSP/TAG
15. Richard Montali, DVM, DACVP, DACZM – University of California-Davis, Dept. of Pathology, Microbiology, Immunology
16. Janet Payeur, DVM, PhD – Head, Mycobacteria and Brucella Section, USDA / APHIS / VS, National Veterinary Services Laboratories
17. Dennis Schmitt, DVM, PhD, DACT – Professor, Southwest Missouri State University
18. Stephen Scott, DVM – Veterinarian, The Elephant Sanctuary
19. Denise Sofranko, DVM – Field Specialist for Elephants, USDA/APHIS/Animal Care
20. Ray Waters, DVM, PhD – USDA / ARS - National Animal Disease Center

## **Appendix II - Abbreviations and Definitions (as they apply to TB)**

Refer to the Guidelines for the Control of Tuberculosis in Elephants for additional definitions.

Culture positive elephant - an animal from which *M. tuberculosis* or *M. bovis* is cultured from any body site or specimen. A culture positive elephant is considered positive until 1) it has completed six months of treatment with documentation that adequate TB drug serum levels have been achieved on two separate testing dates and 2) it can be demonstrated that trunk wash cultures (obtained according to procedures outlined in the Guidelines) on at least two consecutive months are negative.

Direct test – a test that detects the TB organism (e.g. MTD, culture)

ELISA – Enzyme linked immunoassay; a test used to detect and measure either antigen or antibody.

False positive – a test result that wrongly identifies an animal as infected

False negative – a test results that wrongly identifies an animal as uninfected

First line TB drugs: isoniazid, pyrazinamide, rifampin, ethambutol, and streptomycin

Indirect test – a test that detects a response to the organism (e.g. antibodies) but not the organism itself

MAPIA – MultiAntigen Print Immunoassay; a serological assay in which antigens are applied to a nitrocellulose membrane by micro-aerosolization (printing), followed by antibody detection using standard techniques

MTB complex – consists of *M. tuberculosis*, *M. bovis*, *M. bovis BCG*, *M. africanum*, *M. canetti*, and *M. microti*

Multi-drug resistant (MDR) TB – a strain of TB resistant to two or more of the first line TB drugs

MTD (Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test<sup>®</sup>) – Direct target-amplified nucleic acid probe test for the in vitro diagnostic detection of *Mycobacterium tuberculosis* complex rRNA in acid-fast bacilli (AFB) smear positive and negative concentrated sediments from sputum, bronchial specimens, or tracheal aspirates. FDA approved direct test for smear positive and negative specimens. MTD in smear-positive human patients has a sensitivity of 96.9% and a specificity of 100%, PPV of 100%

and NPV of 87.5%. MTD in smear-negative human patients has a sensitivity of 72% and a specificity of 99.3%, PPV of 94.7% and NPV of 95.3%.

PCR – polymerase chain reaction; a nucleic acid amplification technique in which specific sequences of DNA are replicated, allowing for detection of target sequences that otherwise would not be present in high enough numbers to be detected.

Rapid Test – a quick diagnostic test for TB that uses a mixed grouping of selected mycobacterial antigens

RFLP – restriction fragment length polymorphism. A technique used to determine the particular strain of TB

Sensitivity – a measure of the ability of a test to identify infected animals (true positives)

Specificity - a measure of the ability of a test to identify non-infected animals (true negatives)

### **Appendix III – links to Guidelines for the Control of Tuberculosis in Elephants (2003)**

<http://www.aphis.usda.gov/ac/TBGuidelines2003.pdf>

[http://www.elephantcare.org/protodoc\\_files/new2004/TB\\_Guidelines\\_2003\\_Final.pdf](http://www.elephantcare.org/protodoc_files/new2004/TB_Guidelines_2003_Final.pdf)

## Appendix IV - Elephant Tuberculosis Bibliography (www.elephantcare.org)

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