

**RECOMMENDATIONS FOR THE DIAGNOSIS, TREATMENT AND
MANAGEMENT OF TUBERCULOSIS (*Mycobacteria tuberculosis*)
IN ELEPHANTS IN HUMAN CARE**

**Prepared by the Stakeholders Task Force on
Management & Research Priorities of Tuberculosis in Elephants**

Executive Summary

African and Asian elephants are both susceptible to infection by *Mycobacterium tuberculosis* (Mtb) complex organisms. Since 1996, *Mycobacterium tuberculosis* (Mtb) has emerged in the United States as a disease primarily of Asian elephants (*Elephas maximus*). The Asian elephant has lived in close association with humans in Asian range countries for thousands of years; elephants have been working animals, as well as spiritual and cultural icons. This close partnership is likely responsible for the exposure of the Asian elephant to a disease for which humans are considered the primary reservoir. Incidental reports of Mtb-like disease in the Asian elephant go back thousands of years (Chalke 1962). However, the confirmation of Mtb in elephants has only occurred very recently and is the result of the application of modern veterinary medicine and diagnostics. A decade and a half of routine testing and monitoring in the United States has taught us a great deal about Mtb in elephants. However our understanding of its epidemiology and pathogenicity in elephants is still evolving (Feldman 2013).

In general, Mtb is transmitted through close, prolonged contact with a person or animal that is shedding the organism. Therefore, transmission of elephant Mtb to humans is more likely an occupational health concern than a general public health concern. Prevalence studies from 1997-2011 have shown an Mtb point prevalence of 5.1% in the living captive U.S. Asian elephant population. For the same time period, the point prevalence of Mtb in captive African elephants in the U.S. was 0% (Feldman 2013). Although African elephants are not immune to infection with Mtb complex disease, Mtb is very rare. Moreover, most infected elephants do not become clinically ill and are diagnosed as a result of routine trunk wash cultures or at necropsy. However, clinical experiences with Asian elephant Mtb infections have shown that disease manifestations vary widely from serious and even fatal disease to subclinical infection.

There are multiple diagnostic and screening tools available to assist in the diagnosis of Mtb in an Asian elephant, but confirming the diagnosis of true clinical disease remains challenging. All of the methods have some value and should be utilized as appropriate. In addition new tests being developed will need validation. The best and most reliable method currently available for identifying infected animals is the routine culture of trunk wash (TW) samples which is considered the gold standard of diagnosis. The trunk wash (TW) is the elephant equivalent of a human sputum sample. Serological tests, while useful for screening purposes, identify animals that may have been exposed but not infected or animals that may be infected but not shedding. The available serologic tests have not been validated and should not be used as solo diagnostic tests. However, serologic tests results should be considered along with the elephant's entire medical history and may lead the attending clinical veterinarian to increase diagnostic surveillance. The recommendations presented here do not support the imposition of animal isolation, treatment or travel restrictions based solely on serologic testing results. Instead they strongly support the professional experience of the attending clinical veterinarian in determining any testing, management and or treatment regimen.

Research into the epidemiology of Mtb in elephants is ongoing and our understanding of Mtb improves with each case. Nevertheless, many years of study will be needed to completely understand Mtb in the elephant. One consistent aspect of the disease has emerged, though, that the risk of transmission of elephant Mtb appears to be through close, prolonged contact with an infected animal that is shedding the organism. Thus, Mtb infection in elephants should be treated as soon as a facility can do so. If well managed and treated, Mtb infection in an elephant does not pose a threat to elephant caretakers, the general public or other animals.

This document is a multi-year effort of the Elephant Care Stakeholders (hereon “the stakeholders”) made up of veterinarians, elephant managers, public health specialists, epidemiologists, pharmacologists, human physicians and other professionals working with elephants in zoos, circuses, and private facilities. These efforts were initiated upon a recommendation from APHIS/Animal Care at the U.S. Department of Agriculture (USDA), to bring more transparency and stakeholder involvement into the process of developing useful, consistent and easy to follow guidelines for dealing with elephant tuberculosis. The stakeholders offer these **‘Recommendations for the Diagnosis, Management, and Treatment of Tuberculosis in Elephants’** with the intent that they will be a useful guide for veterinarians, elephant managers, and public health officials dealing with elephants and will serve as an accurate source of information for those groups and the general public. These recommendations are meant to serve as a living document to be updated regularly as the science and knowledge of Mtb in elephants advances through good management, medical surveillance, and scientific cooperation. The Stakeholders will continue to work closely with the USDA, State Veterinarians, and State Public Health Veterinarians and other officials to identify research priorities, learn more about potential risks and Mtb transmission pathways to further refine these recommendations for diagnosis, management, and treatment of tuberculosis in elephants.

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Background Information on the Elephant

Although this document provides extremely specific information on one particular disease of elephants, the authors feel some general background on elephants is warranted for the interested reader.

Currently, two species of elephants exist, African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*). Most male and female African elephants have tusks that grow continuously throughout their lifetime. As a rule, only the bulls have large tusks in Asian elephants, but in some countries, many males do not have tusks at all. Female Asian elephants have very small tusks called tushes which may be barely visible. Both species are housed in zoos, circuses and private facilities around the world, and both species have a long history of association with people. In general, African elephants that were brought to the United States were wild-caught animals, whereas Asian elephants, brought in prior to being listed as an endangered species under the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) during the 1970s, were purchased from camps in Southeast Asia where they were cared for by mahouts. Many Southeast Asian countries have a high prevalence of human tuberculosis; thus, older Asian elephants may have been exposed to human tuberculosis very early in their lives. The imported wild African elephants are unlikely to have had this early close human and Mtb exposure.

In human care, elephants can be handled using a variety of techniques. These include unrestricted contact, in which the caretaker and the elephant share space, and restricted contact, in which the caretaker and elephant are separated by bars or the elephant is on tethers while the caretaker is in shared space. Most facilities use a combination of techniques that depend on the facility, the experience of the caretakers, the elephants' training and personality, as well as the gender of the elephant. This is relevant to the diagnostic and treatment sections of this document because no matter what management style is utilized, the facility needs to be able to test and treat the elephants in their care as needed.

Much is unknown about elephants. The great size of the animals precludes many basic medical techniques such as auscultation with a stethoscope or radiographs of the thorax and abdomen. Moreover, their physiology is extremely unusual, and extrapolations from cattle and horses with regard to pharmacology, physiology, and pathophysiology often fail. This likely represents the ancient evolutionary history of elephants, a species with a greater than 50-million year lineage, and few close living relatives. Questions about diagnosis and treatment of an otherwise well-known disease are often thwarted by a complete lack of information about basic elephant physiology, including immunology, organ function, and drug metabolism. The upshot of all these unknowns is that basic research into general aspects of elephant health is greatly needed and would be excellent avenues for further research.

African elephants are a threatened species and Asian elephants are listed as endangered. Their survival even into the next millennium is uncertain. Rampant poaching for ivory is decimating wild African elephant populations. Asian elephants continue to be poached and killed during human-elephant conflicts. Habitat-loss and disease is affecting both species of elephants in the wild. It is of absolute necessity that we provide our North American elephants with the best medical care based on science, and using evidence-based medicine to guide decision making rather than political

ideology or misconceptions. The future of elephants is uncertain and conservation efforts in the wild and in captivity will be needed to help ensure we continue to share the planet with these unique and wondrous creatures. This document was composed with that mission in mind.

Frequently Asked Questions

What is tuberculosis?

Mycobacterium tuberculosis (Mtb) is a bacteria that causes most cases of tuberculosis in elephants. It is primarily a human disease that can cause serious respiratory disease and other types of illness in people.

How is tuberculosis transmitted?

Tuberculosis is not easily transmitted as it typically requires prolonged, close contact with a person or animal that is shedding the bacteria. The bacteria can be aerosolized by the infected person or animal. If these bacteria are inhaled, they can cause disease in another person or animal. In healthy people, infection with Mtb often causes no disease, since the person's immune system either kills or “walls off” the bacteria. This walled-off state of bacteria is considered a latent infection. Further, only about 5 to 10% of latently infected persons will develop active TB disease. The relationship between infection, latency and disease is unknown in elephants.

How do I know if an elephant has tuberculosis?

The best method to definitively confirm that a living elephant has an Mtb infection is to find the Mtb organisms during routine culture of trunk wash (TW) samples, lung lavage, biopsy or other fluids excreted by the elephant. Other ancillary tests, such as serological tests, may be used to help support a diagnosis or but are not definitive. Definitive diagnosis in a dead elephant can only be confirmed upon necropsy of the animal, using recommended protocols and submitting appropriate samples for mycobacterial culture.

What is a trunk wash?

A trunk wash (TW) represents a sample from an elephant's lower respiratory tract and is the equivalent of human sputum sample. Because of their anatomy, elephants can't cough. Instead, they are trained to blow hard into a specimen container. A complete description of a TW can be found in Appendix 3. The triple TW technique refers to a series of three TW done within a 7 day period. The procedure requires no sedation or undue stress to the animal nor any specialized or expensive equipment.

Is there a validated diagnostic serological test confirming Mtb infection in elephants?

At this time, serologic tests have only been validated in known populations and cannot be used to confirm an Mtb infection in the general elephant population. Serologic tests can be used by the

attending clinical veterinarian to decide if increased TW culture surveillance for an individual or group of elephants is appropriate.

Have fomites been shown to be a means of transmission of Mtb spread between elephants or from elephants to other animals?

To date, fomite transmission, acquiring an Mtb infection from an inanimate object such as clothing, or tools to an animal has not been proven as a means of spreading Mtb between elephants or people (Volgenest 2013).

How often does a clinically healthy elephant need to be tested for tuberculosis?

It is recommended that each elephant with no prior exposure or history of Mtb disease be routinely tested by receiving three trunk washes within a 7 day period once a year, (the triple TW technique). An elephant is categorized as risk level A if it has no history of infection with Mtb or exposure to an Mtb infected elephant, or other animal within a 5 year period.

How often do I need to trunk wash (TW) test an elephant while it is being treated for tuberculosis? The current recommendation of this document for testing a confirmed Mtb positive elephant is a single TW culture once a week for the first 2 months of treatment, followed by a triple TW culture monthly throughout the treatment period. After treatment is completed triple TW cultures can be performed every other month for 2 years to confirm success of treatment.

What is the minimum recommended distance an Mtb-infected elephant should be housed from other elephants? Currently the degree of exposure, distance and time between a confirmed infected elephant and other elephants required to transmit Mtb is unknown. In order to reduce the chances of transmission between animals, the infected animal should be treated as soon as a facility can make plans to do so and thereby stop the shedding of the organism into the environment. Additionally the facility should address ventilation, hygiene and sanitation protocols so that they are designed to reduce aerosolization and contamination within the facility. A facility may decide on a case-by-case basis whether an infected animal should be isolated during the entire time of treatment, isolated only temporarily, or kept with the herd while treatment is undertaken.

Can you get tuberculosis from riding an elephant, or visiting a circus or zoo elephant exhibit?

Brief incidental contact, such as might occur as part of an event where members of the general public are allowed to ride, feed, touch or view an elephant, would be extremely unlikely to result in an exposure. In general, Mtb is transmitted through close, prolonged contact with a person or animal that is shedding the organism and therefore, transmission of elephant Mtb to humans is more likely an occupational health concern than general public health concern. To date there have been no known verified transmissions of Mtb between elephants and humans that did not fit the model of prolonged, close contact with an infected animal or aerosol contamination (Murphree et al 2011).

Elephants are traveling into my state; what precautions should be taken to prevent the transmission of Mtb to my livestock? No special precautions are needed. For elephants to travel they must be routinely screened and found negative by TW methods. There has never been a documented case of Mtb transmission from elephants to livestock.

Frequently Asked Questions for State and Regulatory Veterinarians

Elephants may travel across state lines for facility transfers and exhibitions. The issue of Mtb in elephants has generated a great deal of discussion and confusion. Misunderstandings are common about the different tests, the risks, if any to livestock and to the general public as well as how to appropriately evaluate a group of elephants or an individual elephant coming into a region or state. The following section addresses these questions.

What paperwork should accompany an elephant(s) when they enter a state?

The elephant(s) must be accompanied by a Certificate of Veterinary Inspection (CVI) written by an accredited USDA Class II veterinarian and dated within 30 days of arrival into that state. Many zoological facilities utilize a form provided by the AAZV (see Appendix 6). Other facilities use a generic or livestock CVI form from the state of origin. Either is acceptable. The CVI should include the name, age and gender of each elephant in the group, the dates of any vaccinations, if given, and the date that the triple trunk wash (TW) series was performed. For Category A animals, one triple TW series must have been done within one year. For Category B animals, a triple TW series must be done quarterly from the time that the elephant(s) was placed in Category B. The veterinarian writing the health certificate should list to which category each elephant on the certificate belongs.

In addition, elephants over the age of five should travel with copies of at least two years of TW results. These should list the laboratory where the TW was cultured, the name of the elephant, and that they are final results. The results should state that each TW was negative.

What are the differences between a Category A, Category B and Category C elephant?

Elephants in Category A have no known exposure to culture positive animals within a 5 year period. These elephants are negative on TW and have no clinical signs. They are tested by the triple TWseries technique once a year. Elephants in Category B may have had contact with an Mtb positive animal within 5 years. These animals are negative on TW but are undergoing increased surveillance by having TW performed quarterly rather than annually. There are no recommended travel restrictions for Category B elephants as they are being well screened and monitored. Category C animals are TW positive (have had Mtb organisms isolated by mycobacterial culture from a TW sample) and are considered infected. These elephants would only travel if necessary for medical care.

What if the elephant is TW negative but reactive on a serological test (DPP, MAPIA, STAT-PAK or ELISA?)

Many elephants fall into this group. If the elephant has two years of annual triple TW cultures, it should be considered negative. The serologic tests are not diagnostic and have never been validated in an unknown Mtb status population of elephants and should not be used for regulatory purposes.

Elephants are traveling to a livestock arena or venue in my state, what precautions should be taken to prevent the transmission of Mtb to other livestock?

No special precautions are needed. For elephants to travel they must be routinely trunk washed and found negative by culture. There has been no documented case of Mtb transmission from elephants to livestock.

Is there a public health threat from riding or feeding an elephant, or from visiting a circus or zoo elephant exhibit? Brief incidental contact, like one may have as part of an event where members of the general public may be allowed to ride, feed or touch an elephant, would not likely result in an exposure. In general, Mtb is transmitted through close, prolonged contact with a person or animal that is shedding the organism and therefore, transmission of elephant Mtb to humans is more likely an occupational health concern than a general public health concern. (See Appendix 5 Comments from National Association of State Public Health Veterinarians, NASPHV to USDA)

For public health and human health concerns and questions, consult with your state public health veterinarian.

Have fomites been shown to be a means of transmission of Mtb spread between elephants or from elephants to other animals? To date, fomite transmission has not been proven or documented as a means of spreading Mtb between elephants or people. The routine removal of feces, urine, bedding and hay and sanitation of animal barns and areas such as a livestock arena should be practiced between livestock shows or other animal exhibitions as a matter of basic hygiene for these facilities. The basic sanitation practices routinely employed between livestock shows are appropriate for use following an exhibition involving elephants.

Diagnostic Approach

Monitoring for Mtb is part of a standard preventative medicine program for elephants. The clinical veterinarian should establish a preventive medicine program through monitoring of the individual elephant's condition and the herd's health status. Routine preventative exams and diagnostics will allow the establishment of normal baseline data for each animal, the detection of early disease, and treatment monitoring. The attending veterinarian should look at the relevance of each testing modality and make a complete assessment of the situation to aid in disease risk analysis. The veterinarian of record can then plan for appropriate testing, science-based interpretation of data, and a focused, evidence-based treatment plan in each unique situation.

An overall approach for Mtb diagnosis, similar to that used for any disease workup, includes:

1. Obtaining a complete history (elephant and herd),
 - Review of anamneses including general condition and Mtb-specific problems:
 - The individual animal's medical history
 - Species involved (Note: In the United States, Mtb disease has been found exclusively in Asian Elephants for the past ten years)
 - Previous history, origin/location of animals
 - Travel history and potential Mtb exposure at each location
 - Current problems (including age-related diseases)
 - Physical examination (including body condition score) and weight
 - The herd's medical history
 - Previous history of problems
 - Training for medical procedures
 - History of Mtb monitoring results
 - Necropsy results of herd mates
 - New acquisitions
 - Review of husbandry practices including proximity/exclusion of other elephants as well as other animal species
 - Quarantine procedures
 - Human Mtb monitoring of animal care staff and changes in test results
2. Determining the general health of the elephant(s).
 - Complete blood count, CBC, blood smear
 - Chemistry panel
 - Urinalysis
 - Acute phase proteins/Protein electrophoresis sent to the University of Miami (Isaza et al 2014).
 - Fecal analysis for parasites
 - Fecal culture for enteric pathogens
3. Serum-banking at -80° C for future diagnostics

4. Targeted Mtb testing. See also Tables I and II, below.

-Mtb-specific testing for the detection of Mycobacterial organisms

Antemortem tests:

- Culture: the gold standard is culture of fluid from a trunk wash (TW)

Other auxiliary samples include expelled mucous, vaginal fluid, tissue biopsy, lung/airway lavage fluid. Samples must be sent to an appropriate laboratory experienced in culturing Mycobacterial organisms

-Acid-fast staining: not specific for Mtb.

-Polymerase Chain Reaction (PCR): cannot differentiate viable vs dead organisms

Postmortem tests

Culture of lung, lymph nodes, or granulomas

Note: The presence of granulomatous lesions is NOT pathognomonic for Mtb. Other Mtb complex species, saprophytic Mycobacteria and other non-tubercular organisms can cause granulomas. Nevertheless, the finding of lung abscesses, lymph nodes abscesses or granulomas of any size warrant an increased index of suspicion (Lacasse et al 2007).

PCR: see comments in antemortem test section

Immunohistochemistry: not routinely performed

-Mtb indirect testing for detection of host immune response

All tests are antemortem tests

Serology: can increase index of suspicion, but both false positives and false negatives can occur

Immune response assays (IGRAs): not yet validated for elephants

Research in this field is ongoing (Landolfi et al 2014)

Tuberculin skin testing (PPD): does not work in elephants.

Table I: Mtb Diagnostic Tests: Direct Testing Methods

Direct Method	Pros	Cons	Availability
Trunk Wash Culture ^a	Positive results are indicative of infection and shedding at time of sampling	False negatives due to technique, anatomy, intermittent shedding, size and activity of lesion. False positives due to laboratory error or contamination at sampling site are possible, although rare. Requires staff and elephant to be trained	Available Special laboratory required
Lung Lavage ^b	Positive results are likely indicative of infection Potential visualization of lesions Potential for superior sample quality compared to trunk wash from reduced contamination	False negatives are possible Requires staff training and veterinary expertise False positives due to laboratory error are possible, although infrequent Expensive equipment Sedation and analgesia required	Severely limited and not routinely performed
Post-mortem tissue culture ^c	Positive results are indicative of infection	False positive results due to laboratory error are possible, although rare Does not provide ante-mortem results	Available
PCR	Potentially highly specific Potential for sampling multiple tissues: trunk wash, post-mortem sampling Faster turnaround time compared to culture	Does not establish active infection Limited validation; full spectrum of disease not included False positives due to laboratory error are possible Requires staff and elephant to be trained for ante-mortem sampling False negatives possible Requires appropriate handling and transport of samples	Experimental

^a Isaza and Ketz, 1999^b Hildebrandt personal communication^c http://www.aphis.usda.gov/animal_welfare/downloads/elephant/Postmortem%20Exam%20Procedures.pdf and http://c.ymcdn.com/sites/www.aazv.org/resource/resmgr/imported/eleph_research_and_necropsy_protocol_august_2012.pdf**Table II: Mtb Diagnostic Tests -Indirect Testing Methods**

Indirect Method	Pros	Cons	Example	Availability
Serology	<p>Potentially quick turnaround.</p> <p>Potential for monitoring response to treatment</p> <p>Comparatively convenient; some tests can be performed stallside.</p>	<p>Limited validation; full spectrum of disease not included.</p> <p>May indicate exposure and immune response but does not confirm infection or shedding</p> <p>History of use as diagnostic, rather than screening tool for regulatory purposes</p> <p>Incompletely documented sensitivity and specificity which has not been validated in general elephant population</p> <p>Regulatory use has restricted practitioner access and required supervised blood collection</p> <p>Immune response can be highly variable</p> <p>Requires elephant to be trained for blood draw.</p>	<p>ELISA 6-antigen^{a,b}</p> <p>STAT-PAK^{c,d,e}</p> <p>MAPIA^c</p> <p>DPP^e</p>	<p>Not currently</p> <p>Not currently</p> <p>Not currently</p> <p>Limited availability</p>
Cytokine assays ^f	<p>Potentially quick turnaround</p> <p>Potentially specific for cell-mediated response</p> <p>Potential for monitoring response to treatment</p>	<p>Limited validation; full spectrum of disease not included</p> <p>Requires careful handling of sample, testing within 24 hr;</p> <p>Requires elephant to be trained for blood draw.</p>		Experimental
Gamma interferon ^g	<p>Potentially same pros as serology</p> <p>Cell-mediated response based</p>	<p>Incomplete validation with a full spectrum of disease states</p> <p>Potentially time-limited testing requirements</p>		Experimental
BTB (blood test for tuberculosis) ^h	<p>Potentially same pros as serology</p>	<p>No validation with a full spectrum of disease states</p>	Note: developed for cervids	Not currently
Intra-dermal tuberculin test ^b		<p>Requires recheck 72 hours later</p> <p>Poor test sensitivity and specificity in elephants</p>		Available but not recommended

^a Larsen et al 2000, ^b Mikota et al 2001, ^c Bontekoning et al 2009, ^d Verma-Kumar et al 2012, ^e Greenwald et al 2009, ^f Landolfi et al, 2014, ^g Angkawanish et al 2013, Griffin and Buchan 1994

Recommended Testing Requirements and Mtb Risk Categories for Elephants **(See also Table III below)**

Testing requirements for elephants vary according to what risk group they belong. Elephants are placed into one of three groups depending on their risk of being positive for Mtb. Testing varies according to risk group.

Category A: These elephants are low risk animals that have had no known exposure to an Mtb culture-positive animal within the past five years. They are also consistently negative by annual triple trunk wash (TW) technique. These animals are tested once a year by the triple TW technique. No restrictions are recommended on their movement from state to state or from facility to facility.

Category B: These elephants have a moderate risk of becoming positive for Mtb. Category B elephants have had contact with an Mtb positive animal within the past 5 years, but are themselves consistently negative by annual triple TW series technique. These animals require increased monitoring. Thus the triple TW technique should be performed every three months for three years. If all 12 cultures remain negative within the three year period, these elephants return to Category A status, to be tested once annually by triple TW technique. No restrictions are recommended on the movement of these animals from state to state or facility to facility.

Category C: These elephants are positive on TW cultures or culture of other body fluid. These animals cannot travel except for specific medical reasons. They are considered infected with Mtb. Once a positive TW is reported and after treatment begins, the elephant should have one TW once a week for two months to determine the time that shedding stopped. If shedding does not stop within an 8 week period, the organism should be re-cultured and sensitivity rechecked. Drug levels should also be rechecked to make sure appropriate levels are being reached. If the facility is having difficulty medicating the animal, and lack of compliance is the cause of the continued shedding, then reevaluation of handling and treatment techniques will be needed.

If the elephant does cease shedding during the first 8 weeks of treatment, then for the duration of treatment, the triple TW series technique should be carried out every two months. At the end of treatment, the animal should be tested by the triple (TW) series every three months for 18 months. No travel is permitted during this time unless for medical reasons. If all TW are culture negative during this 18-month period, the elephant reverts to Category B, and must be tested by triple TW technique every three months for three years. No travel restrictions are recommended for Category B elephants. If the elephant remains negative throughout these three years, it again becomes a Category A elephant, though the veterinarian may decide to continue increased surveillance TW frequency for an indeterminate amount of time.

Table III. Elephant Risk Categories:

Risk categories are determined by the animals' exposure history, treatment history and the results of trunk wash cultures. The serological tests are ancillary tests considered appropriate for clinical use in guiding the attending veterinarian, but not appropriate for regulatory use. Thus, they are not used here to determine risk category.

Category of risk	Exposure to TB	Clinical signs	Test	Frequency of Testing	Status change
Low Risk (Category A)	No known exposure to culture positive animals within 5 years. Negative by culture for Mtb by triple TW technique.	None	TWC	Three times within one week once a year	Elephant remains Category A if TWC stays negative and no other elephant in the herd becomes positive. Elephant becomes Category B if another elephant in the herd becomes positive by TWC
Moderate Risk (Category B)	Contact with TB positive animal within 5 years. Negative by culture for Mtb by triple TW technique.	None	TWC Other ancillary diagnostic tests to these may include tuberculosis serology, CBC/Chemistry, and other tests as determined by the veterinarian of record.	Three times within one week, performed 4x/year	Elephant returns to Category A if culture negative for 3 years Elephants become Category C if culture becomes positive at any time.
High Risk (Category C)	Positive on TWC	N/A	TWC	Single TWC once a week for 2 months. Then TWC every month during treatment, then every three months for two years following treatment.	If TWC stays negative for 2 years after treatment, elephant becomes Category B.

*TWC = Trunk wash culture

Considerations Once an Elephant Cultures Positive on a Trunk Wash (TW).

If an elephant tests positive for Mtb via TW, the diagnostic testing laboratory will contact the attending veterinarian. Because an elephant that is TW positive is considered infected with Mtb, a number of actions are required although reporting requirements will vary by state:

1. Notifications of regulatory personnel
 - The state veterinarian
 - The state public health veterinarian
 - Local public health organization
 - The local USDA VMO veterinarian for the facility
2. Notification within the facility
 - Management
 - Staff working with the elephant
 - State public health officials who will make recommendations & coordinate staff testing
3. Confirmation of the positive result. The notifications listed above must be performed even before confirmation is obtained. If trunk wash (TW) samples have been banked, a banked sample can be sent for repeat culture. A new TW sample may also be warranted.
4. Antimicrobial sensitivities of the positive culture should be requested of the diagnostic laboratory
5. Spoligotyping or complete genetic sequencing of the culture should be requested of the diagnostic laboratory. If the laboratory is not able to perform either spoligotyping, or genetic sequencing the culture should be shipped to NVSL, Ames, Iowa for genetic identification. See contact information for NVSL in appendix 4.
6. Testing of herdmates. All elephants that are herdmates of the positive animal are now moved to Category B. Category B requires quarterly triple TW to be performed on all current herdmates from this point.
7. Review of the positive animal's movement history and notification of other facilities as appropriate.
8. Education of staff on the use of personal protective equipment (PPE) as per the public health officials' recommendations.
9. Discussion within the facility regarding the management of the infected elephant with respect to quarantine, cleaning, and handling
10. Purchase of antitubercular drugs. Because ordering drugs in adequate quantity can take a while, even though susceptibilities will not be immediately available, the facility should investigate sources and costs.

11. Elephant health screening for the infected animal prior to starting treatment. Banking of additional serum samples for potential future research requests should be considered; see Diagnostic Approach starting on page 9.

Considerations in Treating Elephants for Mtb

The primary issue in assessing whether any antitubercular therapy is appropriate for elephants is that there is currently no way to determine its efficacy or success ante-mortem. In humans, clinical improvement can be monitored and confirmed with chest radiographs. In horses, improvement in granulomatous pneumonias can be monitored by sequential ultrasounds of the lungs. None of these techniques are viable options in elephants. First, TB-infected elephants rarely demonstrate outward signs of illness. Second, they are too large to successfully image by any currently available modality.

Finally, the classic signs of Mtb in humans; chronic cough and night sweats are not typically seen in elephants. Elephants are physically incapable of coughing, they only have sweat glands around their feet and weight loss is extremely rare in infected elephants except in very advanced cases of the disease.

From an evidence-based perspective, three things can be monitored in the treatment of Mtb in elephants; first, whether or not the animals are shedding Mtb organisms, information that can only be obtained from TW cultures; second, measurement of serum concentrations of antitubercular medications which can be compared with human clinical breakpoints, and finally, identification of drug-induced adverse effects.

Prevention of shedding is the first goal of treatment. Treating so that the animal does not have serious adverse events associated with antitubercular drug therapy should be the second. Determining appropriate serum concentrations, corresponding doses, frequency and duration of treatment remains empirical in elephants.

Mtb in humans is typically treated with a four drug regimen consisting of isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (ETH). Pharmacokinetic studies in elephants have not evaluated necessary blood concentrations needed for cure, only the amounts of drugs that need to be administered to achieve blood concentration similar to those reported in humans. (Maslow et al 2005a; Maslow et al 2005b; Zhu et al 2005; Peloquin et al 2006).

Serious adverse events associated with antitubercular drugs are well-documented in both humans (Thompson et al 1995; Papastavros et al 2002; Yee et al 2003; Younossian et al 2005; Saukkonen et al 2006) and elephants, although the signs in elephants are akin to those seen in domestic farm animals, not those of humans. (Wiedner and Schmitt 2007; Wilson et al 2010). These adverse events include depression, colic, inappetance and black manure (Wiedner and Schmitt 2007). This suggests that human serum concentrations are or can be toxic for elephants. Lowering the doses of these drugs while keeping target concentrations above the human breakpoint may decrease the incidence of toxicity without compromising effectiveness.

Although elevated liver enzymes are often seen in elephants undergoing treatment for Mtb, it is currently not possible to monitor hepatotoxicity in elephants as it is in humans. This is because hepatocellular enzymes (AST, SDH, GGT, LDH, ALP) tend to vary greatly in specificity, and have not been evaluated in elephants (Boyd 1988). Liver function tests, LFTs which are the gold standards in assessing hepatotoxicity, have also not been validated in elephants. Bile acids, the most commonly used veterinary LFT cannot be used in elephants because the species does not produce bile acids, only bile alcohols. Bile alcohol tests are not currently available.

Another concern in treating elephants with Mtb is determining the appropriate frequency of therapy. No data is available to show that one particular dosing regimen works better than another in elephants. From the perspective of stopping shedding, increased frequency of initial treatment would seem applicable to elephants; however, any of the antitubercular drugs have post-antibiotic effects that last for days, which suggests that an intermittent schedule could also be used (Davies and Neurmberger 2008).

Humans are typically monitored for two years after completion of antitubercular treatment. Recurrence (also called relapse) rates in humans using a 4 drug approach range from 0 to 27% (Lambert et al 2003). Recurrence is considered different from re-infection. Distinguishing between recurrence and re-infection requires molecular techniques. In elephants, there are no published studies indicating the rate of either recurrence or re-infection. While some researchers have suggested the use of serology to monitor post-treatment recovery, validation for this technique is lacking.

Goals of Evidence-Based Antituberculosis Therapy for Elephants

1. Prevent the infected elephant from shedding
2. Prevent the elephant from becoming ill from treatment
3. Achieve serum levels above the breakpoint for relevant drugs
4. Treat daily with four drugs initially then decrease to intermittent therapy
5. Treat for an adequate period of time
6. Intensively monitor TW cultures for next two years, See Category C elephant above

Recommended Treatment Protocol

Treatment of culture-positive elephants can be done in two phases: **initiation phase** and **continuation phase**. The goal of the initiation phase is to rapidly decrease large bacterial populations without creating resistance. During this phase, 3 or 4 drugs are used concurrently, one of which is isoniazid (INH). Isoniazid is responsible for early rapid killing of Mtb organisms often within a few days of starting treatment and will stop shedding. This in turn makes the animal non-contagious to those around it, both animal and human. Other drugs that can be used during this time are ETH (Ethambutol), PZA (Pyrazinamide) and RIF (Rifampin). RIF is a sterilizer that can resolve cavitory lesions. ETH and PZA are symbiotic with the other drugs and important in preventing failure of treatment due to resistance. Quinolones, such as levofloxacin are used in human Mtb treatment if INH and/or RIF resistant strains are present. In animals, enrofloxacin has been used. The initiation phase requires frequent administration of drugs, 5 treatments within a seven day period using all 3 or all 4 drugs. This phase lasts for 8 weeks or 40 doses.

The goal of the second phase of treatment -- the continuation phase -- is continued bacterial kill. Drug numbers are reduced to 2 to 3 drugs concurrently, and frequency of administration is decreased to 3 treatments a week. The continuation phase lasts 64 weeks and consists of 192 doses.

Some elephants cannot tolerate the high intensity initiation phase and show signs of drug toxicity including depression, hard, scant, fetid manure, severe blepharospasm, ocular tearing, and/or weakness. Elephants showing such signs should be given a break from therapy to recover, then started on a **combination regimen**, which is used from beginning to end of therapy. For this regimen, three drugs are used at a lower frequency but at twice the doses used ordinarily. A combination regime lasts 72 weeks and consists of 216 doses. In yet another subset of elephants, the double doses will also cause signs of toxicity to develop. There are few guidelines for such situations, and it is the recommendation of the authors of this document that in such cases, the veterinarian of the facility should consult with pharmacologists and elephant veterinarians to determine how best to proceed.

Adverse effects, either those described above or others, should be reported to the Food and Drug administration (FDA) Center for Veterinary Medicine, (CVM). The stakeholders would also appreciate being made aware of side effects and toxicity associated with treatment so that these treatment recommendations can be amended.

Table IV. Schedule of treatment for Mtb Culture Positive Elephants: Adapted from ATS 2003.

Phase of therapy	Goals	# drugs to use	Frequency	Duration of Phase	# of doses
Initiation	Decrease large bacterial populations. Decrease risk of developing resistance	3 or 4 concurrently with one drug to be INH*	5 treatments per week	8 weeks	40 doses
Continuation	Continue bacterial kill	2 to 3 concurrently	3 treatments per week	64 weeks	192 doses
Combination	For use in elephants that show signs of drug toxicity during the initiation phase described above	3 drugs at 2x the doses described in the previous section	3 treatments per week	72 weeks	216 doses

INH = isoniazid

Table V: Starting drug doses for treatment of elephants with Mtb

DRUG	1° OR 2°	ROUTE	DOSE**
Isoniazid (INH)	Primary	Oral or rectal	2 mg/kg
Rifampin (RIF)	Primary	Oral	10 mg/kg
Ethambutol (ETH)	Primary	Oral	15 mg/kg
Pyrazinamide (PZA)	Primary	Oral or rectal	20 mg/kg
Enrofloxacin (ENRO)	Secondary	Oral or rectal	15 mg/kg

Primary drugs are mycobacteriocidal firstline drugs that are considered necessary for the successful treatment of Mtb. Secondary drugs are considered adjunct drugs to be used in combination therapy. Selection of two primary or first line drugs are recommended for successful combination antitubercular therapy.

There are reports of elephants that have been infected with strains of Mtb that are INH resistant. Here too, there are no clear recommendations for treatment, and each case should be handled individually in consultation with pharmacologists and veterinarians with experience treating Mtb positive elephants.

Table VI: Routes of administration*

Route	Technique	Advantages	Disadvantages	Comments
Oral	Animal asked to open mouth. Entire dose administered either as tablets or mixed in solution.	Not painful.	Animals may refuse or spit out medication or hold meds in their mouth without swallowing.	A bite block can improve compliance. Appropriate for PZA, ENRO, RIF, ETH, and INH.
Rectal	Drug is dissolved in water and administered into the rectum via dosing syringe & tubing.	Can result in increased absorption. Efficient administration of drugs.	Not all drugs can be given by this route although RIF and ETH can be formulated specifically for this route by altering pH.	Manure should be manually evacuated from the rectum prior to administration. Appropriate for PZA, ENRO, INH
Injectable	Drugs are administered intramuscularly with a needle and syringe.	Can confirm that entire dose was given.	Painful. Causes muscle damage and associated with a risk of abscess formation.	Not recommended for elephants long term. May be appropriate for short-term use in specific situations. Possible for ENRO.

* All routes of administration require prior training of the elephant

Recommended Monitoring Protocol During Treatment and Immediate Aftermath:

1. Submit a single trunk wash (TW) for culture every week for the first two months of treatment, then a triple TW once a month until treatment is completed.
2. If any TW are culture positive during treatment, recheck sensitivity of the organism and spoligotype to assess if a new isolate is present. Depending on clinical observations, the following options can be considered.
 - a. Increasing the amount of INH per dose
 - b. Adding in a fifth drug
 - c. Swapping any drug other than INH for another drug

Sourcing medications

Medications can be purchased in bulk for treatment. Samples of new batches of bulk drugs should be tested for purity and for measurement of drug activity levels. Samples can be submitted to the Infectious Disease Pharmacokinetic Laboratory at the University of Florida. Contact: Dr. Charles Peloquin at peloquinlab@cop.ufl.edu.

It is recommended that in addition to being tested for purity, purchase be made from a licensed pharmacy and certified pharmacist compounder. Several facilities have had to obtain permission from the FDA to import large quantities of antitubercular drugs for their elephants.

Evaluation of therapeutic drug levels

Drug levels can be measured in serum or plasma. Plasma is advantageous as it does not require waiting for clotting to occur and centrifugation. Samples should be collected in Lithium heparin tubes. Plasma samples can be submitted to Dr. Charles Peloquin at peloquinlab@cop.ufl.edu. It is recommended to contact Dr. Peloquin prior to testing for specifics of timing, sample collection, and shipping.

Blood levels of all antitubercular drugs should be measured after the first two weeks of therapy. If levels are appropriate, a second drug level measurement should be done after six months of treatment to ensure that drug levels remain adequate. If levels are inappropriate, the drug levels should be altered, and then rechecked after the elephant has been on the new dose(s) for two weeks.

Treatment must be based on culture and sensitivity results. Recent evidence indicates that there is individual variability in the pharmacokinetics of the different antitubercular drugs. Pharmacokinetics also vary according to the route of administration (oral vs rectal) Thus, a modified pK curve is needed for each animal under treatment (Brock et al 2014).

Listed below are starting points for therapeutic drug monitoring (Brock et al 2014).

Table VII: Therapeutic antitubercular drug monitoring in elephants

Drug	Route	Time to sample for Tmax*
Rifampin	Oral	6-8 hours
Rifampin	Rectal	Cannot be given rectally
Isoniazid	Oral	2-3 hours
Isoniazid	Rectal	Approximately 30 minutes
Pyrazinamide	Oral	2-4 hours
Pyrazinamide	Rectal	0.75 to 2 hours
Ethambutol	Oral	1-3 hours
Ethambutol	Rectal	Cannot be given rectally
Enrofloxacin	?	?

*Tmax = time after administration of a drug when the maximum plasma concentration is reached;

Animal Management

Priorities in managing an Mtb infected elephant include optimizing the elephant's health and well-being while preventing the transmission of Mtb to uninfected elephants, other animal species and animal husbandry staff. Management strategies range from disease monitoring to treatment. In rare circumstances, isolation of the infected animal or humane euthanasia may need to be considered. The treatment and management plan must be developed in full consideration of each facility's policies and professional best practices.

Elephant-to-elephant transmission appears to have occurred at some facilities. The lack of certainty with regard to exact mode(s) of transmission of Mtb in elephants suggests the need for basic bio-containment precautions.

Bio-containment and reduction of Mtb spread in the environment.

The development of reasonable bio-containment procedures is specific to each facility. The veterinarian of record and elephant care staff should work together to formulate a plan to physically manage an infected elephant. The plan should be reviewed with the state veterinarian and public health officials. Bio-containment procedures should be instituted as soon as possible. Starting an Mtb-infected elephant on treatment as soon as bacterial susceptibilities are reported reduces exposure of conspecifics and human staff to Mtb organisms. Although fomites are not considered a risk factor for transmission, caution dictates that waste, bedding and substrate should be removed from the bio-containment area and handled in a manner that limits other animal and human exposure. Food, hay and substrate for other animals must be stored away from and outside of all bio-containment areas.

Management of an Mtb infected elephant

Once an Mtb infection has been confirmed in an elephant by trunk wash (TW) culture, that elephant should not travel unless the transportation event is part of the plan for the elephant's housing and medical treatment. The attending veterinarian should confer with state regulatory and public health veterinarians with regard to monitoring and surveillance results prior to release from travel restrictions.

The preferred method of management of an Mtb positive elephant is to prevent/restrict direct physical contact between it and uninfected elephants until it can be verified that the infected elephant is confirmed as not shedding Mtb organisms. But elephants are highly social animals and may suffer psychologically if kept in complete isolation from herd mates. Therefore, isolation may include co-housing of the Mtb infected elephant with a long term conspecific(s). The likelihood of transmission to conspecifics can be greatly reduced by keeping the animals in outdoor, open air as opposed to confined air space. Where indoor confinement is necessary, assuring very high air turnover through an appropriate filter system will reduce exposure. If complete physical isolation of an infected elephant is elected, care should be taken to provide sufficient enrichment, training opportunities and staff interaction. The veterinarian may consider allowing direct social interactions with conspecifics once treatment is being received reliably, drug blood levels have been evaluated, and repeated TW cultures during the treatment regimen remain negative. If isolation is not possible, limiting or eliminating shared confined air space by increasing air flow and ventilation within barns is recommended. Treatment of companion herd mates prophylactically is not recommended as it may increase the likelihood of selecting resistant strains of Mtb.

Elephant care staff management

All staff working in areas of suspected/known Mtb organism contamination need to utilize proper personal protection equipment, PPE respiratory protection (N-95 or higher rated) masks at all times compliant with OSHA regulations. PPE use should be continued until determined that occupational risk has been resolved through consultation with local public health officials. See Occupational Health Section, page xx.

Disinfection of premises

Appropriate disinfection of barn areas housing an Mtb-infected elephant should be practiced. Organic material should be removed from all areas prior to the use of a disinfectant. General cleaning practices should minimize the creation of aerosolized droplets within indoor or enclosed spaces. High pressure hosing should be avoided (Murphree 2011). Apply a phenolic or other mycobacteriocidal disinfectant according to label directions. Where footbaths are present, a mycobacteriocidal disinfectant capable of killing Mycobacteria with some organic load present should be used. Footbaths should be cleaned and maintained daily.

The Center for Food Security and Public Health (CFSPH) publishes guidelines that review disinfectants effective against mycobacterial species. These guidelines can be found at www.cfsph.iastate.edu/Disinfection/index.php

Transmission of Mycobacteria tuberculosis

Human to human transmission of Mtb

Mtb is a well studied disease in human medicine and human-to-human transmission parameters are comparatively well defined. In humans, Mtb is transmitted through close prolonged contact with another person that is shedding the organism.

According to Heyman 2008: “Transmission is affected by exposure to tubercle bacilli in airborne, aerosolized droplet nuclei that measure 1-5 microns in diameter, and are produced by persons with pulmonary or high respiratory tract tuberculosis (e.g., laryngeal) during forceful expiratory efforts (e.g., coughing, singing or sneezing). The droplet nuclei are inhaled by a vulnerable contact into the pulmonary alveoli. Here, the aerosolized particles containing *M. tuberculosis* are ingested by alveolar macrophages, initiating a new infection. The risk of exposure and subsequent infection is linked with the intimacy and duration of the contact, the ventilation in the shared environment, and the degree of contagiousness of the index case.”

Indirect contact with a person who is shedding the organism is unlikely to result in transmission. In an enclosed space, the likelihood of human to human transmission of Mtb is influenced by air volume, exhaust rate, time and circulation. In large indoor settings, because of diffusion and local circulation patterns, the degree of proximity between contacts and the index patient can influence the likelihood of transmission. Other subtle environmental factors (e.g., humidity and light) are not considered relevant to transmission. The volume of air shared between an infectious TB patient and contacts dilute the infectious particles, although this relationship has not been validated entirely by epidemiologic results.

While there is no specific definition of prolonged contact in human to human transmission of Mtb, the likelihood of infection depends on the intensity, frequency, and duration of exposure. For example, airline passengers who are seated for ≥ 8 hours in the same or adjoining row as a person who is contagious are much more likely to be infected than other passengers and only these people would be contacted by public health investigators for follow up in a known Mtb (+) exposure scenario. (National Tuberculosis Controllers Association 2005)

Other routes of Mtb exposure in humans have not been documented or are not considered significant. Furthermore, the airborne route of transmission means that fomites are not an issue and do not require special handling. (Heymann 2008)

Elephant to elephant transmission of Mtb

Initial infections of elephants with Mtb have been hypothesized to come from exposure to infectious humans but no direct well documented evidence exists to confirm this suspicion. Mtb infection however has not been documented as a disease of wild elephants without close human contact. *Mycobacterium bovis* infections have been documented in wild African elephants and in one captive African elephant in the United States (Payeur 2002). Mtb organisms have been transmitted between elephants within the same herd based on the genetic relatedness of the Mtb organisms isolated, but transmission does not seem to be efficient or routine. To date no study has documented how elephant-to-elephant transmission has been effected, but aerosol droplet and prolonged exposure similar to human-to-human transmission are presumed to have occurred since the affected animals were typically long term companions, shared the same barn, and had trunk-to trunk-contact.

Fomite transmission has been postulated in some cases, but no conclusive evidence has been found (Vogelnest 2012).

Occupational vs. Public Health Considerations:

In general, Mtb is transmitted through close, prolonged contact with a person or animal that is shedding the organism. This is different from *M. bovis* which is much more contagious. With Mtb, incidental, more indirect contact with a person or animal that is shedding the organism is unlikely to result in transmission. Therefore, transmission of elephant Mtb to humans is more likely an occupational health concern for people who manage the day-to-day care of elephants rather than a general public health concern. In the United States, facilities that maintain elephants may offer opportunities for members of the general public to touch, feed or ride an elephant. These opportunities are typically offered in such a way that members of the general public do not have prolonged contact with elephants in an enclosed space. Therefore, such contacts would be unlikely to constitute a public health risk. This is particularly true with elephants that are routinely screened and monitored for their Mtb status via routine trunk wash (TW) cultures.

Studies investigating transmission risk to people working with Mtb positive elephants have identified certain occupational risk factors for acquiring disease. These include routine elephant handling, routine elephant training and/or participating in or being present at an elephant necropsy involving an Mtb positive animal (Michalak et al 1998; Oh et al 2002; Murphree et al 2011; Lecu and Ball 2011). In one study, the risk of skin test conversion was increased for elephant caregivers and administrative personnel working in the barn housing the elephant or in offices connected to the barn. Husbandry practices that aerosolized Mtb organisms and delayed and inadequate infection control likely contributed to transmission. (Davis 2001; Murphree et al 2011; Oh et al 2002; Montali et al 2001; Mikota and Maslow 2011; Michalak 1998, National Tuberculosis Controllers Association 2005; Vogelnest 2013, Lecu and Ball 2011)

Investigators have made efforts to determine the number of workers whose tuberculin skin test, (TST) converted from negative to positive while working from weeks to months in a barn with a culture positive elephant. However, controlled epidemiological studies that evaluate the risks associated with working with infected elephants are lacking. Until such studies are reported, it is reasonable for elephant keepers and others who routinely work with elephants to monitor their tuberculosis status and to use personal protective equipment (PPE) designed to decrease the likelihood of tuberculosis exposure when working with an infected elephant. See Occupational Health recommendations which follow.

Although there is no specific definition of prolonged contact between a person and an elephant, investigations suggest that several hours or more of exposure to an infected elephant is likely necessary to result in a human health impact. More specifically, one study found that employees at a facility who spent ≥ 4 hours in the quarantine barn within a year where an untreated culture positive elephant was housed had a greater risk of developing a TST conversion. Barn cleaning practices at this facility promoted aerosolization of bacteria. Greater risk was also incurred by employees in an adjoining building which shared unfiltered airspace with this barn. (Murphree et al 2011). Another study found that people who experienced a TST conversion were those who had spent at least 10 hours within an elephant enclosure housing an Mtb culture positive elephant. (Stephens et al 2013).

Occupational Health Recommendations

Managers and veterinarians of facilities that maintain elephants should consult with their state's State Public Health Veterinarian, and are encouraged to develop an occupational health program in consultation with these occupational health experts. It is recommended that such protocols take into consideration the species of elephants maintained by the facility, the health and diagnostic testing history of those elephants, and the kind of contact members of the general public may have with those elephants. Such protocols should include procedures for:

- Routine tuberculosis screening of employees who work with elephants. All relevant staff should be assessed annually and before beginning to work with elephants in order to help protect both animal and human health
- Employees with acid-fast positive sputum smears. These individuals should not work directly with elephants until it is determined whether their lab findings represent infection with an organism of the *M. tuberculosis* complex.
- Routine education of staff in zoonotic disease prevention
- Education of staff on diagnostic tests or clinical symptoms consistent with active human tuberculosis infection
- Infection control and routine hygiene and sanitation practices including guidelines to reduce direct and indirect aerosol transmission of Mtb.
- Training of employees in the use of PPE.

Directors of facilities maintaining elephants should consult with both their State Veterinarian and State Health Department for guidance in regard to animals and human tuberculosis reporting requirements. A complete listing of State Public Health Veterinarians and State Epidemiologists can be accessed via the Council of State and Territorial Epidemiologist's website within the "points of contact" information at www.este.org. Disease reporting laws vary by state (National Association of State Public Health Veterinarians 2005, Montali et al 2001).

Resources for Occupational Health and Safety Information:

- The Occupational Safety and Health Agency (OSHA) tuberculosis guidelines. OSHA has regulations for recording and reporting tuberculosis infection acquired in the workplace. These are outlined at <http://www.osha.gov/SLTC/tuberculosis>.
- The Centers for Disease Control and Prevention (CDC) *Guidelines for Preventing the Transmission of Mycobacterium tuberculosis in Health-Care Settings* in 2005. The CDC guidelines for preventing transmission of Mtb are available at <http://www.cdc.gov/mmwr/PDF/rr/rr5417.pdf>. Though not specific to elephant care settings, they contain invaluable recommendations and guidance for workers exposed to a source of tuberculosis.
- The CDC document *Biosafety in Microbiological and Biomedical Laboratories* published by the U.S. Department of Health and Human Services in 2007. This document outlines best practices for the safe conduct of work in biomedical and clinical laboratories and animal facilities in regards to *Mycobacterium tuberculosis* and is available at <http://www.cdc.gov/od/ohs/biosfty/bmb15/BMBL>.
- The CDC/National Institute for Occupational Safety (NIOSH) respirator guidance. The CDC/NIOSH Health Respirator Trusted-Source Information is located at

http://www.cdc.gov/niosh/npptl/topics/respirators/disp_part/RespSource.html. This website provides information on appropriate respirator types and usage.

- Iowa State Center for Food Security and Public Health (CFSPH) disinfection guidelines. These guidelines review disinfectants including those effective against mycobacterial species and can be found at www.cfsph.iastate.edu/Disinfection/index.php
- *Compendium of Veterinary Standard Precautions for Zoonotic Disease Prevention in Veterinary Personnel*. These guidelines were developed by the National Association of State Public Health Veterinarians in response to a growing recognition of the occupational risks inherent in veterinary practice and the need for infection control guidance for veterinarians. They are available at <http://www.nasphv.org/documentsCompendia.html>

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maximus). In: AAZV, AAWV Joint Conference, South Padre Island, Texas 2010;170.

41. Yee D, Valiquette C, Pelletier M, et al. Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. *American journal of respiratory and critical care medicine* 2003;167:1472-1477.

42. Younossian AB, Rochat T, Ketterer JP, et al. High hepatotoxicity of pyrazinamide and ethambutol for treatment of latent tuberculosis. *European respiratory journal* 2005;26:462-464.

43. Zhu M, Maslow JN, Mikota SK, et al. Population pharmacokinetics of pyrazinamide in elephants. *Journal of veterinary pharmacology and therapeutics* 2005;28:403-409.

Appendix 2.

Epidemiologic Definitions:

There are a number of epidemiologic terms that are applicable to any infectious disease, and that must be understood to facilitate sound clinical decision-making and application of Mtb treatment and management measures. It is imperative that the clinician consider what is known about both the epidemiology of Mtb and the diagnostic test modalities available. Below are a few definitions for understanding diagnostic test interpretation and TB disease epidemiology.

Mtb Direct Tests: Tests that determine the presence of *Mycobacterium tuberculosis* in the sample. These tests can determine viable organisms (e.g., culture) or potentially non-viable components of the organism, such as DNA fragments (e.g., PCR) or proteins.

Mtb Indirect Tests: Tests that measure or detect an animal's immune response to *Mycobacterium tuberculosis*.

Sensitivity: A measure of the ability of a test to identify infected animals. Sensitivity is the frequency of a positive or abnormal test result (e.g., a test that is outside of the reference interval) when a disease is present (i.e., the percentage of true positive results). $\text{Sensitivity} = [\text{TP} \div (\text{TP} + \text{FN})] \times 100$ where TP = true positive; FN = false-negative). Validation of test sensitivity requires inclusion of a full spectrum of disease states. Test sensitivity may vary among populations. No *Mycobacterium tuberculosis* diagnostic test is 100% sensitive.

Specificity: A measure of the ability of a test to identify non-infected animals. Specificity is the frequency of a negative or "normal" test result when a disease is absent (i.e., the percentage of true-negative (TN) test results). $\text{Specificity} = [\text{TN} \div (\text{TN} + \text{FP})] \times 100$. Validation of test specificity requires inclusion of a full spectrum of disease states. Test specificity may vary among populations. No *Mycobacterium tuberculosis* diagnostic test is 100% specific.

Negative Predictive Value: A numerical value for the proportion of individuals with a negative test result who have the target condition (i.e., the probability that a person who is a test negative is a true negative.) This probability is relevant to determining the usefulness of a test when applied to animals of unknown disease status, and is clinically more important than test sensitivity and specificity. The negative predictive value of diagnostic tests can be low in populations with high disease prevalence.

Positive Predictive Value: A numerical value for the proportion of individuals with a positive test result who have the target condition (i.e., the probability that a person who is a test positive is a true positive.) This probability is relevant to determining the usefulness of a test when applied to animals of unknown disease status, and is clinically more important than test sensitivity and specificity. The positive predictive value of diagnostic tests can be low in populations with low disease prevalence.

Risk analysis: The phases of a risk analysis, according to World Organization for Animal Health (OIE) Code, include hazard identification, risk assessment, risk management, and risk communication. Data for conducting formal risk analysis for tuberculosis in elephants is limited,

although qualitatively considering the parts of formal risk assessments and risk analyses is useful for managing the risks of tuberculosis.

Risk assessment: The process of evaluating the likelihood of exposure, infection, or spread of a disease. This is a part of formal risk analysis. The parts of a formal risk assessment include release, exposure, and consequence assessments, as well as risk estimation. Data for conducting formal risk assessments for tuberculosis in elephants is limited, although qualitatively considering the parts of formal risk assessments and risk analyses is useful for assessing the risks of tuberculosis.

Trunk Wash (TW) Culture: A direct test designed to detect viable Mtb organisms via culture of material obtained from a trunk wash. It is a practical method of obtaining a culture sample from a large proportion of the elephant population. The procedure requires no sedation or undue stress to the elephant. Additionally, the procedure requires no specialized or expensive equipment. The recommended routine Mtb monitoring is an annual triple mycobacterial trunk wash culture. Each testing event should consist of three independent collections on three days within a one week period. It is recommended that food and water be withheld from elephants for 2 hours before the TW is performed to help minimize the contamination of the TW sample.

Mtb Infected Elephant: An elephant from which one positive Mtb culture has been isolated from a bodily discharge or lesion.

Appendix 3.

The Trunk Wash Technique for Routine Surveillance and Diagnosis of Mtb in Elephants (Isaza and Ketz 1999)

Summary

A trunk wash is a practical method of collecting a sample from an elephant's distal respiratory tract for Mycobacterium culture. The procedure, however, is potentially dangerous to the handlers and requires cooperation of the elephant. Because of the limitations of using culture results as a screening test, the trunk wash results should be interpreted with care. A positive culture result identifies an elephant that is shedding tuberculosis organisms whereas a negative result is non-diagnostic.

Materials and methods

The trunk wash technique requires that the elephant allow the handlers to restrain and manipulate the tip of trunk. This is difficult in an untrained elephant in that most elephants resent this manipulation, and the trunk is many times stronger than the combined force of several handlers. It is therefore important that the animals be trained to present the trunk, allow gentle manual restraint, and manipulation of the trunk tip during the collection of the sample. The training period varies with the individual elephant, the prior behavioral conditioning of the animal, and the skill of the handlers. In our experience, most animals can be adequately trained for the procedure in 2-4 weeks.

Materials Needed:

- Sterile 0.9% saline solution
- Sterile 60 ml syringe
- 1 gallon plastic zip lock type bags (heavy duty)
- 50 ml, screw top, plastic jar or centrifuge tube

As long as attention is given to collecting a clean sample from the distal nasal passages, the materials and techniques for the sample collection can be modified. For example, some clinicians prefer to use a 14-gauge red rubber tube feeding tube inserted into the trunk tip instead of simply flushing the sterile saline into the trunk tip. Another common variation is to use a sterile plastic container to catch the trunk wash fluid instead of a plastic bag.

Procedure

A routine screening of an elephant should consist of a series of three trunk wash samples collected on separate days within a one-week period, i.e. triple trunk wash series or collection. Trunk washings should be collected in the morning and prior to water being offered to the animal, or after food and water has been withheld for a minimum of 2 hours. These recommendations are made in an attempt to obtain a representative sample of the nasal flora from the previous night, and to avoid the dilution effect caused by elephants drinking water with their trunks.

The elephant's trunk is manually restrained by the handlers so that the tip is held up. The 60 ml syringe filled with sterile saline is then inserted into one of the nostrils and the saline quickly flushed into the trunk. The handler then lifts the trunk tip as high as possible to help the fluid flow as far into the trunk as possible. The 1 gallon plastic bag is then slipped over the trunk tip and the tip of the trunk is lowered to allow the fluid to drain. If possible, the elephant is allowed to exhale into the bag during this collection phase of the procedure. A good sample should retrieve a significant portion of the saline that was placed into the trunk (about 40 ml). The sample should contain visible mucus from the inside of the trunk and often contains dirt and food particles that are normally found inside the trunk. The collection of moderate amounts of foreign material does not invalidate the sample. If, however, the collector feels the contamination is excessive, a second flush may be attempted.

Once the sample is collected in the plastic bag, it is carefully transferred into a labeled container. Ideally, the sample is refrigerated and sent directly to a laboratory for processing and mycobacterial culture. If the sample cannot be sent directly for culturing, it may be frozen in a regular freezer (-20 to -10 °C) until it can be sent to the laboratory. Often the recommended three daily cultures samples are collected and frozen until all samples are collected and the batch of samples can be sent to the laboratory together.

The trunk wash as a method of collecting a culture sample from elephants has become the standard method of screening elephants for Mtb. It is a practical way of obtaining a culture sample from a large proportion of the elephant population. The procedure requires no sedation or undue stress to the animal. Additionally, the procedure requires no specialized or expensive equipment.

An important consideration of this procedure is that it can potentially be very dangerous to the handlers. This is particularly true when attempted on an uncooperative elephant, because any attempts to manually restrain the trunk in an uncooperative elephant can lead to injury. The time spent training the elephant to accept this method will greatly increase the efficiency and safety of the procedure. In some cases, with potentially dangerous or unpredictable animals, an increased level of handler safety can be obtained by having the animal lie in sternal or lateral recumbency prior to sample collection. This technique does not guarantee safety or successful sample collection, as it still requires cooperation of the animal and does not replace adequate training. In the case of elephants managed under protective contact, the animal's trunk can be handled through a set of bars. This method still requires that the animal is fully cooperative and, therefore, usually requires extensive training prior to the collection.

A second safety issue is the potential for zoonotic infection. Recently there has been documentation of a zoonotic transmission of tuberculosis between humans and elephants (3). During the collection of the trunk wash sample, there is exposure to aerosolized mucus from the elephant's respiratory tract. The authors, therefore, suggest that the collectors and handlers wear PPE of that includes an N-95 or greater particle mask. Minimal precautions would include a well fitted respirator or face mask capable of filtering 0.3 micron particles, disposable gloves, and working in a well-ventilated, sunlit, area.

Mycobacterial culture as the primary method of detecting infected animals has several limitations that are best illustrated by examination of the underlying biological assumptions. The first assumption is that most infected elephants have respiratory infections. Although the literature suggests that most infected elephants have respiratory infection, there have been no comprehensive necropsy studies to confirm these observations. The second assumption is that most infected animals shed mycobacterial organisms into the respiratory tract. There is little data that determines if and when an infected animal will begin shedding organisms. It is unknown what proportion of elephants can carry latent or “walled off” infections that would be missed with culturing techniques. A third assumption is that animals that are shedding will pass mycobacteria organisms at least once in the three-day testing period. Currently it is unknown if shedding animals pass organisms periodically or continuously. Finally, the samples collected from the distal trunk are often contaminated with normal bacterial flora and foreign material. It is assumed that these contaminants do not routinely overgrow or mask the growth of pathogenic mycobacteria, although no studies have tested this assumption. The interpretations of the culture results should, therefore, be limited. A positive culture is strong evidence that the animal is shedding mycobacteria and is infected; negative culture results provide little information as to whether the elephant is infected or not.

Culturing the distal trunks of all the animals in a population will only detect animals shedding tuberculosis through the trunk, and not detect all animals that are infected. However, with time and repeated cultures of all animals in the population, it may be possible to detect and treat most of the elephants shedding infectious organisms. If these animals are then treated properly and shedding of organisms stops, the spread of tuberculosis from elephant to elephant should decrease in the population

Appendix 4.

Suggested Certified Laboratories for Mycobacteria Cultures.

1. USDA APHIS VS

National Veterinary Services Laboratories (NVSL)

1920 Dayton Avenue

Ames, IA 50010

Lab web site: http://www.aphis.usda.gov/animal_health/lab_info_services/diagnos_tests.shtml

Dr. Suelee Robbe-Austerman

Veterinarian, Mycobacteria and Brucella Section

(515) 337-7837 Fax: (515) 337-7315

Email: Suelee.Robbe-Austerman@aphis.usda.gov

50 ml conical screw-top leak-proof centrifuge tubes are preferred and available free of charge from NVSL.

Send trunk washes to NVSL either frozen or on icepacks by overnight express (Federal Express handles diagnostic samples). Containers should be leak proof and double-bagged. If lesions are submitted for culture, tissues should be frozen and sent on ice packs overnight. Lesioned tissues should be split and ½ should be sent to the histopathology lab so PCR can be run to see if the tissue is compatible with tuberculosis. There is no charge for histopathology on lesioned tissue.

Use the VS Form 10-4 for submission. If the formalinized tissue is sent separately from the frozen tissue, please indicate on the submission forms that there are 2 separate packages coming from the same animal so that the reports can be combined and accession numbers coordinated when they reach NVSL. It is also helpful to call or email NVSL contacts when sending sample from Mtb suspects to schedule testing and relay any relevant history of the case.

NVSL Trunk wash cost: \$98 per sample for processing which includes a Gen Probe® DNA probe on any isolate. If the sample is positive for mycobacteria and speciation is requested, the charge is \$122.00 per sample which includes biochemical analysis, 16s rDNA sequencing analysis, spoliotyping and VNTR genotyping. DNA fingerprinting of *M. tuberculosis* or *M. bovis* isolates is also available. Antimicrobial susceptibility testing is available for *M. tuberculosis* complex organisms for \$112.00 per isolate. Please contact NVSL at (515) 337-7388 for test schedule.

Do we need to explain spoligotyping, VNTR genotyping, and DNA fingerprinting? Yes, we probably do.

To establish an account at NVSL for billing, contact **Connie Osmundson** (515) 337-7571 or Email: Connie.J.Osmundson@aphis.usda.gov .

(User fees as of December 1, 2014). Call lab before shipping samples for current prices and schedule of testing or check prices at the NVSL web site:

http://www.aphis.usda.gov/animal_health/lab_info_services/diagnos_tests.shtml

2. Mycobacteriology Laboratory at National Jewish Medical and Research Center

National Jewish Medical and Research Center

1400 Jackson St.
Denver, CO 80206
(303) 398-1384

Manager Clinical Laboratories:

Jamie Marola, MB(ASCP)
National Jewish Health Advanced Diagnostic Laboratories
303.270.2479 Office
303.398.1339 Laboratory
720.290.2204 Mobile
303.398.1953 Fax

Clinical Laboratory Supervisor:

Kimberly Sue Messina, MT-ASCP
Mycobacteriology Lab
Room K422a
Lab Phone: 303-398-1339
Office Phone: 303-398-1347
Cell Phone: 469-323-1352

For price list; sample collection and shipping instructions and requisition form:

<http://www.nationaljewish.org/research/diagnostics/adx/labs/mycobacteriology/requisitions-and-specimen-handling.aspx>

3. Your State Public Health Laboratory other CDL certified Laboratory.

Appendix 5. NASPHV comments to the USDA June 28, 2012



NATIONAL ASSOCIATION of STATE PUBLIC HEALTH VETERINARIANS, INC.

June 28, 2012

Janet B. Payeur DVM, MPH, PhD
Chair, USAHA Elephant Tuberculosis Subcommittee
Scientific Outreach Coordinator
National Veterinary Services Laboratories
1920 Dayton Avenue
Ames, IA 50010

Dear Dr. Payeur:

On behalf of the National Association of State Public Health Veterinarians (NASPHV), I am providing summary comments on the April 2012 Draft Revision of *Guidelines for the Control of Tuberculosis in Elephants*. These comments represent a compilation of concerns and requests from a subcommittee of members, who share a particular interest and public health experience in responding to tuberculosis in elephants.

1) Description under airborne transmission: Comments received from tuberculosis control physicians in two separate state health departments expressed concerns about the inclusion of the sentence, "Microorganismsmay be dispersed over long distances by air currents and may be inhaled by susceptible individuals who have not had face-to-face contact with (or been in close proximity to) the infectious animal or person (Siegel 2007). The likelihood of a person (or animal) to inhale a sufficient dose of *Mycobacterium tuberculosis* carried by wind currents in an outdoor space, or semi-open well ventilated enclosure is deemed to have extremely rare potential for an exposure to occur. This description in the Guidelines needs to qualify that airborne transmission can occur in closed spaces with a shared ventilation system that may communicate with animal areas and areas housing or officing personnel. The Siegel reference is describing airborne transmission in an enclosed area, not one that is ventilated to outside air.

2) Definition of public contact: The current definition, i.e., "any situation", is too broad to be applied for the purposes of classifying potential human exposures and managing risk of zoonotic transmission. We request that the definition be modified to reflect what is known about zoonotic transmission rather than to be ultra-conservative and cause unwarranted fear or concerns. What is known is that the greatest risk of spread of *M. tuberculosis* from an infectious elephant to a human requires frequent exposures within close proximity to an infectious elephant, or sharing of a common airspace in an enclosed area under conditions of repetitive aerosolization of droplet nuclei (reported in EID 2011;17:366-371). Brief, incidental contact in or around an infected elephant would not likely result in an exposure; nor would just touching an elephant. The scientific evidence for fomite transmission to a person also needs to be presented if included in this definition as a potential route of exposure.

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NASPHV, June 28, 2012

Similar to what is provided in section #13, we suggest added language in the definition of public contact that states something on the order of: "Consult with the State Public Health Veterinarian or State Epidemiologist in your state to obtain guidance regarding the public health risks an infected elephant may pose to the general public as the particular circumstances involving a TB-infected elephant are variable."

3) Page 10, Group 2: Request adding more detail on what is defined as "cessation of exposure". For previously culture and STAT-PAK negative elephants in a herd with a culture positive elephant undergoing treatment, we assume exposure cessation has occurred six months after the TB-infected elephant has been undergoing approved anti-tuberculosis treatment, or six months following effective isolation of the TB-infected elephant, or six months following removal of the TB-infected elephant from the premises. If the subcommittee is in agreement with these parameters, they need to be clearly stated in the Guidelines.

What is the basis for the added 6 month travel/public contact restriction (as compared to 2010 Guidelines) for elephants in Group 2? This restriction seems appropriate for travel that would involve change in ownership, i.e., relocation to a new facility/herd, but seems unnecessary for performing elephants. Since *M. tuberculosis* has a latency period, and travelling elephants do not typically comingle with those outside of their herd or come into frequent direct contact with the public, this addition appears to be over restrictive and should be more granular (and science-based) to types of travel. Elephants in this group would be very unlikely to pose a risk of zoonotic transmission to the general public.

4) Request insertion of a small paragraph towards the end of Section #7, TB Management Groups 1-4 (page 15): NASPHV requests consideration of some added language to the Guidelines that addresses working with local and/or state public health officials in assessing public health and occupational health risks associated with a particular elephant. Proposed language -- "Facilities who maintain elephants falling into Groups 2-4 should be prepared to share information, including but not limited to, employee TB status and TB risk factors, elephant medical history, elephant housing and activities with public health officials in order to assist in any assessment or contact investigation associated with the public health risk of an elephant(s).

5) Page 17, section #3, Group 4 elephants: This section is written from the perspective that the STAT-PAK and MAPIA tests are 100% sensitive and specific for elephants that are infected with *M. tuberculosis*. We know of elephants that have been *M. tb* culture positive, but negative upon subsequent and repeated STAT-PAK and MAPIA testing. This needs to be addressed for Group 4 elephants. It is an unnecessary expense to the elephant owner to continue to require the serological testing once it is well demonstrated that the individual culture positive animal is non-reactive on these tests, and the MAPIA test will have no value in assessing successful treatment status or recrudescence of infection.

6) Page 18, Section B, Quarantine without treatment: We recognize there is limited data available on the "*M. tb* elephant-to-elephant transmission range" whether by aerosol transmission or theoretical fomite transmission; however, USDA in collaboration with USAHA must develop a beginning standard distance between a culture positive elephant and other

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NASPHV, June 28, 2012

elephants for these guidelines to be operational. Simply stating, "Quarantined elephants should be kept out of range from non-infected animals..." is insufficient. Would 100+ yards (> 300 feet) be considered "out of range", > 150 yards?

7) Page 18, Section C, Euthanasia: Suggest clarifying "...showing clinical signs.... Consider changing toshowing clinical signs of progressive and active tuberculosis disease.

8) Page 21, 23, 25: Terms C_{max} and T_{max} are used in the pharmacology sections, but are not defined in Section 2 – Definitions. Suggest adding to definitions before citing in Guidelines.

9) Page 26, Section 12, Postmortem Examination: "It is essential that a post-mortem examination be performed on all elephants that die." Our reviewers requested clarification – does this refer to all elephants regardless of Group 1-4 status, or just elephants that die while being treated for tuberculosis?

10) Page 28, Section 14, Reporting: Although we are in agreement that all positive *M. tb* culture results in elephants should be reported to the State Veterinarian and State Health Department, it is not a correct statement in the Guidelines to simply state, "Tuberculosis is a reportable disease." Disease reporting laws are determined by the individual states. We know that in some states only tuberculosis in livestock animals is required to be reported to the State Veterinarian. Since elephants are not livestock, they are not captured by this reporting requirement in many states. Tuberculosis in humans is reportable to the respective state health department; however, very few states have disease reporting laws that require reporting of tuberculosis in animals to the public health agency. Based on discussions that occurred at a national stakeholders meeting in Fort Worth, TX, in August 2011, there appears to be a misunderstanding among USDA and other elephant-centered organizations about this existing gap in reporting of tuberculosis in elephants.

List of Minor Edits (typographical, formatting, etc.)

Page 3, first paragraph: remove space from NASPHV reference

Page 3, Culture positive contact: ...for *M. tb* (insert space, add "b") complex

Page 5, *M. tb* and *M. tb* complex: Add space between M and tb

Page 11, bulleted footnote under Group 2: Remove bullet; appropriately place footnote – seems out of place and a little difficult to put into context.

Page 11- 14, Follow-up status for Group 2 Elephants after Testing: Reformat – missing "g" at end of testing; all "p" s translating strange in other versions of Microsoft Word

Page 15, second paragraph: Several words have inappropriate spaces, e.g., "levofloxaci n", "IN H". This may be simply a problem with the draft version that is being circulated, but check master copy. Similar problem with "Isoni azid" in third paragraph on page 16.

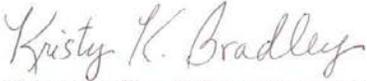
Page 19, last paragraph: Spacing problem again with "pyrazi namide" and "fl uoroqui nolones"

Page 23, Pharmacokinetics: Paragraph is represented as bolded in received draft version. Remove bolding.

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NASPHV, June 28, 2012

Thank you for your thoughtful consideration of NASPHV review and input into the Guidelines. If you have any questions pertaining to our comments, please contact me by phone at 405.271.7637 or by Email at Kristyb@health.ok.gov.

Sincerely,



Kristy Bradley, DVM, MPH, DACVPM
Vice President, National Association of State Public Health Veterinarians
Office of the State Epidemiologist
Oklahoma State Department of Health
1000 NE Tenth Street, Room 606
Oklahoma City, OK 73117

Appendix 6.

Example AAZV CVI filled out for 2 adult Asian elephants with 2 years of TW history included.

AMERICAN ASSOCIATION OF ZOO VETERINARIANS

STANDARD CERTIFICATE OF VETERINARY INSPECTION

No. 99292

OWNER: Bob's Elephant Ranch ADDRESS: 1234 Anywhere Ave PHONE: (123) 123-4567

CONSIGNEE: Joe's Elephant Land ADDRESS: 345 Elephant Uppia Lane PHONE: (314) 123-3456

DATE ISSUED: 01/01/2015 MODE OF TRANSPORT: Land Air Sea

NAME OF AGENT: _____

ANIMAL I.D. Tattoo Band Tag Etc.	NAME Common Scientific	PHYSICAL DESCRIPTION Age Sex Weight Other	HISTORY Date of Vaccination including name of product used Date of last deworming including name of product used Housed with: other animals Recent Health problems with similar or adjacent species Given name and dosage of all drugs given prior to or during transport (including antibiotics, restraint or immobilizing drugs) Tuberculin Used and Dosage <input type="checkbox"/> Mammalian old tuberculin <input type="checkbox"/> Bovine PPD Other (Specify) _____ EIA results for Equine <input type="checkbox"/> Positive <input type="checkbox"/> Negative Brucellosis Test for Ruminants Test Dilution: 1:50 1:100 1:200 Results: _____ Site of injection: _____
Jumbo	Asian Elephant <i>Elephas maximus</i>	45 years old Female 8500 lbs.	Method of Examination: <input type="checkbox"/> Visual <input checked="" type="checkbox"/> Physical Jumbo - MB Category A - Treatments history: Tape TW Jan 3-5, 2015, Lab: NVSL, Results: Neg Tape TW May 15-17, 2014, Lab: NVSL, Results: Neg Tape TW May 15-17, 2014, Lab: NVSL, Results: Neg Mabel - MB Category A Tape TW Jan 3-5, 2015, Lab: NVSL, Results: Neg Tape TW May 15-17, 2014, Lab: NVSL, Results: Neg
Mabel	Asian Elephant <i>Elephas maximus</i>	42 years old Female 7700 lbs.	

Permit Obtained if Required: Yes No

*Notify, as an approved veterinarian, that the above described animals have been inspected by me and that they are not showing signs of infectious, contagious, and/or communicable disease, (except where noted). The vaccinations and results of tests are indicated on the certificate. To the best of my knowledge, the animals listed on this certificate meet the state of destination and Federal Interstate requirements. No further primary quarantine or impound.

Signature: [Signature] DVM National Vet. Accreditation Prog. ID: 1234

Printed Name: Joe Zoo Vet State Veterinary License No. 1235 AK

Approved by: John State Vet State Veterinarian State Permit No. (if applicable): 1234

AAZV Member Non Member

OWNER/AGENT STATEMENT: The animals in this shipment are as certified to and listed on this certificate.

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Appendix 7. Participants

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

Name	Affiliation	Ft Worth TX 8/1- 2/2011	Tulsa OK 7/9- 10/2014	Pittsburgh PA 8/25- 26/2013	Ft Worth TX 8/18- 19/2014
David Abraham DVM	Centre for Ecology and Wildlife Diseases				
Noha Abou- Madi DVM, MSc. DACZM	Cornell University				
Tom Albert	Feld Entertainment				
Jill Allread	Public Communications, Inc				
Lisa Armitige MD, PhD	Heartland National TB Center				
Doug Armstrong DVM	American Association of Zoo Veterinarians				
Kay Backues DVM, DACZM	Tulsa Zoo. American Association of Zoo Veterinarians				
Jason Barr	Fort Worth Zoo				
Colin Basler DVM/MPH	Centers for Disease Control and Prevention				
Henry Boom MD	Case Western Reserve University				
Kristy Bradley DVM, MPH	Oklahoma Department of Health				
Paul Calle VMD, DACZM	AAZV/WCS				
Egeene Daniels DVM	University of North Texas				
Sharon Deem DVM, PhD, DACZM	St. Louis Zoo				
Genny Dumonceaux DVM	Palm Beach Zoo				
Shannon Ferrell DVM	Fort Worth Zoo				
Mitch Finnegan DVM	Oregon Zoo				
Martha Fischer	St. Louis Zoo				
Michael Fouraker	Fort Worth Zoo				
Jennifer Furin MD, PhD	Case Western Reserve University				
Joan Galvin JD	Outdoor Amusement Business Association				
Matt Gombrich MD	Vivian Biosciences				
Hank Hammatt	Elephant Care International				
Terry S. Hensley MS, DVM	Texas Animal Health				
Thomas Hildebrandt DMV	Institute for Zoo and Wildlife Research				
Darryl Hoffman	Houston Zoo/Elephant Managers Association				
Thomas Holt DVM	State of Florida				
Robert Hunter PhD	International Elephant Foundation				
Ramiro Isaza DVM, MPH, DACZM	University of Florida				
Kari Johnson	Have Trunk Will Travel				
Jennifer Landolfi DVM, PhD	University of Illinois				
Scott Larsen DVM, DACZM	Denver Zoo				
Bob Lee	Oregon Zoo				

Nancy Lung DVM	Fort Worth Zoo				
Mike McClure	Maryland Zoo/Elephant Managers Association				
AJ Marlar DVM, MRCVS, DACVO	Fort Worth Zoo				
Joel Maslow MD	University of Pennsylvania				
Bob Meyer DVM	National Assembly of State Veterinarians				
Corissa Miller DVM	Ecotone Wildlife Veterinary Services				
Susan Mikota DVM	Elephant Care International				
Dave Miller DVM, PhD, DACZM	Miller Veterinary Services				
Michele Miller DVM, MS, PhD, DACZM	Palm Beach Zoo		Via Skype		
Thaddeus Miller DrPH, MPH	University of North Texas				
Ted Moskal	Vivione Biosciences, Inc				
Rendi Murphree PhD	Centers for Disease Control				
Julia Murphey DVM, MPH	American Association of Public Health Veterinarians				
Debbie Myers DVM, DACZM	Pittsburgh Zoo				
Meenakshi Nagendran DVM	United States Fish and Wildlife Service				
Justin Oedekoven DVM	United States Animal Health Association				
Francisco Olea-Popelka DVM, PhD	Colorado State				
Florence Olivet-Courtois, DVM	Medicine des Animaux Sauvages et Exotiques				
Deborah Olson	International Elephant Foundation				
Luis Padilla, DVM, DACZM	St. Louis Zoo				
Janet Payeur DVM, MPH, PhD	USDA/APHIS				
Kris Petrini DVM	United States Animal Health Association				
Heidi Riddle	Elephant Managers Association				
Suelee Robbe-Austerman DVM, PhD	USDA/APHIS				
David Sabala DVM	USDA/APHIS Animal Care				
Dennis Schmitt DVM PhD, DACT	Missouri State/Feld Entertainment/RBBB				
Denise Sofranko DVM	USDA/APHIS Animal Care				
Christopher Stremme DVM	Veterinary Society for Sumatran Wildlife Veterinarians				
Ginger Takle DVM, DACZM	Pittsburgh Zoo				
Martha Weber DVM	Saint Louis Zoo				
Ellen Wiedner VMD, DACVIM	The National Elephant Center				
Mark Wilson DVM	Florida International Teaching School				
Erica Wilson DVM	Dickerson Park Zoo				
Ralph Zimmerman DVM	Albuquerque BioPark				