2017 RECOMMENDATIONS FOR THE DIAGNOSIS, TREATMENT AND MANAGEMENT OF TUBERCULOSIS (Mycobacterium tuberculosis) IN ELEPHANTS IN HUMAN CARE

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

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Executive Summary

African and Asian elephants are both susceptible to infection by *Mycobacterium tuberculosis* (Mtb). The Asian elephant has lived in close association with humans in Asian range countries for thousands of years and this close partnership is likely responsible for the exposure of the Asian elephant to a human disease. African elephants, in contrast, with whom historically have had fewer contacts with humans the disease has been less common. Incidental reports of Mtb-like disease in the Asian elephant go back thousands of years, (Chalke 1962). The confirmation of the existence of Mtb infection in elephants has only occurred recently after a testing program was initiated in 1996 in elephant-holding facilities in the United States. *Mycobacterium tuberculosis* (Mtb) is now recognized as a disease primarily of captive Asian elephants (*Elephas maximus*) though some cases have been identified in wild Asian elephants and one case not confirmed by culture in a wild African elephant. Although two decades of routine testing and monitoring in the United States have taught us a great deal about Mtb in elephants, our understanding of its epidemiology and pathophysiology in elephants is still evolving.

In general, Mtb is transmitted through close, prolonged contact with an infected person or animal shedding the organism. Transmission of the disease from elephants to humans is therefore an occupational health concern of elephant caretakers rather 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 and 0% for African elephants (Feldman 2013).

Asian elephants infected with Mtb may have variable disease manifestations but most infected elephants do not show clinical signs unless the disease becomes advanced. Multiple diagnostic and screening tools are available to assist in diagnosis but confirmation remains challenging. Currently the only test available for identifying truly infected elephants is through culture of the organism, the gold standard test for diagnosis. Samples for culture are typically obtained from living elephants using trunk wash samples, considered the equivalent of human sputum samples.

As of October 2015, the United States Department of Agriculture, Division of Animal and Plant Health Inspection Services (USDA APHIS) no longer regulates the surveillance, diagnosis and treatment of the disease in elephants within the United States. Currently, USDA APHIS places this responsibility in the hands of the attending veterinarian. Thus, veterinarians working with elephants need a thorough understanding of the disease in these species and a willingness to work closely with their state veterinarian and public health officials to manage the disease appropriately if diagnosed.

This document is a continuing multi-year effort of the Elephant Care Stakeholders (hereon “the stakeholders”), a group of veterinarians, elephant managers, public health specialists, epidemiologists, pharmacologists, physicians and other professionals with many

years of experience working with elephants in zoos, circuses, and private facilities. Their efforts were initiated following a recommendation from USDA APHIS to bring more transparency and stakeholder involvement to the development of useful, consistent and easy to follow guidelines for dealing with elephant tuberculosis.

The stakeholders offer these **2017 Recommendations for the Diagnosis, Management, and Treatment of Tuberculosis in Elephants in Human Care'** as a guide for veterinarians, elephant caretakers, government regulators and public health officials dealing with elephants as well as an accurate source of information for the general public. This document is a multi-year collaborative effort that is updated regularly as the science and knowledge of Mtb in the elephant advances. The stakeholders advocate good management, medical surveillance, scientific cooperation, and the use of evidence based medicine.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health.

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Background Information on Elephants

Because this document provides extremely specific information on one particular disease of elephants, the authors feel a brief overview of general facts about elephants is warranted.

Currently, two species of elephants exist, African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*). African elephants are generally somewhat larger, with bulls reaching 18,000 to 20,000 lb, and both sexes having tusks. In comparison, only male Asian elephants have tusks, and the largest bulls seldom exceed 16,000 lb. Both species are housed in zoos, circuses and private facilities around the world. Adult African elephants seen in the United States are either wild-caught animals or were born in the US, whereas older Asian elephants in the US were likely purchased from mahouts in Southeast Asian elephant camps. Since many Southeast Asian countries have a high prevalence of human tuberculosis (TB), older Asian elephants may have been exposed to human TB very early in their lives. Imported wild African elephants are unlikely to have had this early close human contact and thus had limited Mtb exposure. Although African elephant importations continue to this day, no further importation of Asian elephants has occurred since the 1970s, when the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) listed Asian elephants as an endangered species. One can safely assume that young Asian elephants living in North America were born here.

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 shares space. Some facilities use a combination of techniques that depend on the facility, caretaker experience, and the elephants' training, personality and sex. In 2014, the Association of Zoos and Aquariums (AZA) mandated that all their accredited elephant-holding facilities utilize restricted contact only. In truth, no matter what management style is utilized, the facility should be able to test and treat their elephants 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 unusual, and extrapolations from cattle and horses with regard to pharmacology, physiology, and pathophysiology often are inaccurate. Thus, although Mtb is a well-known disease in humans and domestic animal species, it remains poorly understood in elephants, a species where little is known about their physiology, immunology, organ function, and drug metabolisms. Basic research into general aspects of elephant health is greatly needed.

African elephants are a threatened species and Asian elephants are endangered. Their survival even into the next millennium is uncertain. Rampant poaching for ivory is decimating both African and Asian elephant populations. Loss of habitat, human-elephant conflicts, and disease are also affecting both species of elephants in the wild. Thus, providing elephants in human care with evidence-based medicine to guide decision-making is a critical need as well as an ethical mandate. The future of elephants is uncertain and conservation efforts in the wild and in human care are needed to ensure we continue to share the planet with these unique and wondrous creatures.

Frequently Asked Questions (FAQ)

What is tuberculosis (TB)?
Tuberculosis is a disease caused by *Mycobacterium tuberculosis* (Mtb), a bacterium. Tuberculosis is primarily a human disease but Mtb can transfer to other species and has caused most cases of TB in Asian elephants. Thus, Mtb is zoonotic.

Are all organisms named *Mycobacterium* of equal concern from a trunk wash culture in regards to TB disease in humans and elephants?
No. Mtb belongs to a group of organisms known as "the Mtb complex". Members of the Mtb complex, which also includes *Mycobacterium bovis* (in cattle and other hoof stock) and *Mycobacterium pinnipedii* (in marine mammals) are contagious, have zoonotic potential, and require treatment. If these organisms are isolated in a culture, the laboratory is mandated to contact the facility to discuss the findings.

Non-Mtb complex mycobacteria however, are NOT of significant concern. Known as "atypical mycobacteria" or "saprophytic mycobacteria," these mycobacteria live in substrate. Examples include *Mycobacterium avium*, *Mycobacterium intracellulare*, and numerous others. Because elephants root around in the dirt, they may get atypical mycobacteria in their trunks, and the final results of a trunk wash culture may mention the identification of such organisms. However no treatment is needed for these organisms if they are reported in culture results. If there is any question about whether a mycobacterial organism is significant or not, the laboratory should be contacted.

How is tuberculosis transmitted?
While a number of factors influence human to human transmission, such as the concentration of tubercle bacilli an infectious person produces, the duration of exposure, proximity to an infectious person and the size of the space associated with the exposure, transmission typically occurs after close, prolonged contact with a person expelling many tubercle bacilli. Mtb transmission in humans is well documented to occur via small (1-5 microns in diameter) airborne particles, called droplet nuclei. Infectious droplet nuclei are generated when persons who have pulmonary or laryngeal TB disease cough, sneeze, shout, or sing. Transmission occurs when a person inhales droplet nuclei containing Mtb organisms and the droplet nuclei traverse the mouth or nasal passages, upper respiratory tract, and bronchi to reach the alveoli of the lungs. Mtb is not transmitted by surface contact. https://www.cdc.gov/tb/education/corecurr/pdf/chapter2.pdf

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. To date, no study has documented how elephant-to-elephant transmission has occurred; aerosol droplet and prolonged exposure similar to human-to-human transmission are presumed to have occurred, because the affected animals were typically long-term companions, shared the same barn, and had trunk-to trunk-contact. To date, all known verified transmission of Mtb between elephants and humans involved facility employees working with or exposed to the housing environment of an infected animal at that facility (Murphree et al 2011, Zlot 2016).

What is the difference between an active infection and a latent infection?
An active infection is one in which the bacteria are growing in the body, and the animal or person is shedding Mtb organisms into the environment. An active infection can be transmitted to another individual. In a latent infection, the bacteria are typically inactive and walled-off by

the immune system. A latent infection cannot be transmitted to another individual, and the person or elephant with a latent infection is neither contagious nor shedding living organisms into the environment. Five to 10% of latently infected people will ever develop an active tuberculosis infection. This reactivation occurs when the body, for some reason such as immune compromise from age, other diseases or stressors, "releases" the walled off bacteria back into the system. The relationships between infection, latency and disease is unknown in elephants.

Can you get tuberculosis from riding an elephant, or visiting a circus or zoo elephant exhibit?
Brief incidental contact as occurs with elephant rides, public feeds, touching or viewing an elephant is extremely unlikely to result in infection. Because Mtb is typically transmitted through close, prolonged aerosol contact with an infected person or animal that is actively shedding the organism, transmission of Mtb from elephants to humans is more of an occupational health concern than a health concern to the general public.

If I am in a barn with an infected elephant, can I spread the disease if I get the bacteria on my clothing or shoes?
To date, this type of transmission, known as "fomite transmission," which refers to acquiring or spreading an infection from an inanimate object such as clothing or equipment is NOT proven as a means of transmitting TB. https://www.cdc.gov/tb/education/corecurr/pdf/chapter2.pdf

How do I know if an elephant has tuberculosis?
One cannot tell from simply looking because elephants with Mtb usually show no signs of disease. The best method to definitively confirm that a living elephant has an Mtb infection is to find the Mtb organisms by culture in a trunk wash 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 of Mtb in a dead elephant can be made if positive culture results are obtained from necropsy samples of the animal.

Is it possible to make a risk assessment about the likelihood of an elephant having Mtb?
Elephants are categorized as Risk Level A, B, or C depending of their history of infection or exposure to other Mtb-infected elephants. This concept is discussed in detail in this document.

What is a trunk wash?
A trunk wash (TW) represents a sample from an elephant's upper and lower respiratory tract and is the equivalent of human sputum sample. Because of their anatomy, elephants do not readily cough. Instead, they can be trained to blow hard through their trunk into a specimen container. A complete description of a TW can be found in Appendix 2. The procedure requires no sedation or undue stress to the animal nor any specialized or expensive equipment, but does require time to train the animal.

How do I test a clinically healthy elephant for tuberculosis?
For an elephant without any prior exposure or history of Mtb disease, the triple trunk wash (TW) technique can be performed once a year. The triple TW technique is done three times over a 7 day period. Collected samples should be submitted for mycobacterial culture to a laboratory specializing in these organisms. If available, qPCR, a real-time Polymerase Chain Reaction test should be requested along with the culture. Veterinarians caring for elephants may also opt to use other tests such as serological tests for herd surveillance.

Are there other tests for tuberculosis in elephants besides the trunk wash?
Tests that use serum/blood to look for exposure to TB are available but cannot be used to confirm TB, although they may be useful for routine herd surveillance in conjunction with culture. A qPCR test is being developed that may be a useful adjunctive test to trunk wash cultures. Several common tests in humans are not possible in elephants. For example a chest radiograph (x-ray) is not possible in an elephant because of their large size, and the skin test, aka the TST using PPD, does not work in elephants. Other tests such as gamma interferon are being developed, but for now are experimental only.

What do I do if an elephant comes up positive by culture on a trunk wash?
The first step is to notify facility staff, public health officials, and the state veterinarian. The next step is to confirm the culture by repeating the trunk wash. Before results are back, however, the facility should start treatment. Treatment is discussed fully later in this document.

Do I need to isolate an infected elephant?
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. In all cases, starting treatment immediately to stop shedding, and improving hygiene and sanitation in the barn are very important.

If I do isolate the elephant, what is the minimum recommended distance an Mtb-infected elephant should be housed from other elephants?
Currently neither this distance nor the amount of time needed to transmit Mtb from an infected elephant to an uninfected one are known. Here too, though, steps can be taken to decrease the risk of disease transmission. First is to start treatment of the infected animal as soon as possible in order to stop shedding of the organism into the environment. Additionally the facility should examine its ventilation, hygiene and sanitation protocols in order to increase the dilution of bacteria and reduce their aerosolization.

How long should an elephant be treated for tuberculosis?
No one treatment protocol has been used in treating all infected elephants. Current recommendations using a one or two-phase protocol recommend 12 months of treatment. Treatment length may be affected by the antibiotics that can be successfully administered to the elephant. Using combination protocols with less frequent dosing or antibiotics of lesser known effectiveness may extend the treatment period for 15 to 18 months. See the treatment section for more details.

How often should an elephant be tested while it is being treated for tuberculosis?
Elephants should be tested throughout treatment. Current recommendations are a single trunk wash culture (and qPCR if available) once a week or full triple TW for the first 2 to 3 months of treatment to verify that treatment is effective in stopping the shed of infectious bacteria, followed by a triple TW cultures obtained monthly until treatment is complete.

How often should an elephant be tested after it finishes treatment for Mtb?
After treatment is completed, triple TW cultures should be performed at least quarterly (4X a year) to monitor and confirm success of treatment. The veterinarian of record may prescribe to do triple TW cultures more frequently.

Frequently Asked Questions for State and Regulatory Veterinarians

Because elephants may travel across state lines for facility transfers, breeding or exhibitions, questions about Mtb in elephants have often arisen from the regulatory sector. This FAQ addresses the different tests, possible risks to livestock or the general public and how to appropriately evaluate an elephant or elephants coming into a region or state.

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 prior to arrival into that state. The CVI can be on a form provided by the American Association of Zoo Veterinarians (AAZV), or a generic or livestock CVI form from the state of origin (see Appendix 3). The CVI should include the name, age and sex 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. The veterinarian writing the health certificate should list to which category each elephant on the certificate belongs (Category A, B, or C). Elephants over the age of five should travel with copies of at least two years of TW results. TW results should document the name of the laboratory, the name of the elephant, that the results are "final" and that each TW was negative. (Note: a description of the trunk wash technique can be found in Appendix 2).

What are the differences between Category A, Category B and Category C elephants?
Elephants in Category A have had no known exposure to culture positive animals within a 5 year period. These elephants are negative on TW and have no clinical signs. Elephants in Category B may have had contact with an Mtb positive animal within 5 years but are negative on all TW. No travel restrictions exist for either Category A or B elephants. Category C elephants are TW positive (i.e., Mtb organisms were isolated by culture from a TW sample) and are considered infected. Category C elephants would only travel if necessary for medical care

Are Mtb testing recommendations the same for all elephants?
No. Category A elephants need only one triple TW series done within a 12 month period. Category B animals should receive triple TW series done quarterly from the time that the elephant(s) was/were placed in Category B. Category C elephants receive considerably more testing which is described elsewhere. See description of Elephant Risk Categories, page 17.

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 negative annual triple TW cultures, it can be considered negative, and going forward from 2017, the qPCR test performed on TW samples), it should be considered negative. The serologic tests are not confirmatory for Mtb and should not be used as a solo test 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. Travelling elephants (both Categories A and B) are routinely trunk washed and negative by culture. There has been no documented case of Mtb transmission from elephants to livestock. There are no documented cases of fomite transmission of Mtb

DIAGNOSTIC APPROACH

Testing for Mtb infection should be part of the preventative medicine program of every elephant-holding facility. This aspect of the preventative medicine program should be reviewed and updated annually by the attending veterinarian.

A. Risk assessments

A standard risk assessment for Mtb infection in an elephant or its herd includes the following:

1. Complete history of the elephant and its herdmates including:

Signalment: Mtb is primarily a disease of Asian elephants although several cases were identified in African elephants in Europe and one unconfirmed by culture report in a free ranging African elephant (Obanda 2013).
Travel and housing history of elephant and herdmates with regard to Mtb exposure
Current health problems (including age-related diseases)
Diagnostic procedures for which the elephant is trained (i.e., TW, blood draw, etc)
History of Mtb complex testing & results over at least a five-year period
Necropsy results of herdmates at both current and previous locations
Quarantine protocols
Recent herd acquisitions
Reproductive history

2. Staff screening program for Mtb (This information is protected by HIPAA laws)

Staff testing protocols should be developed with the input of local public health officials
Staff Mtb risk assessment including:
- travel to countries with high rates of human Mtb
- known exposure to infected (*M.bovis*) animals other than elephants (dairy cattle, deer, etc)
- known exposure to infected family members
- known exposure to infected elephants either at current or previous facilities

3. Husbandry practices that could increase risk of transmission of Mtb

Barn ventilation
Barn cleaning procedures, i.e., use of high-pressure hoses, which can aerosolize bacteria (Murphree 2011)

4. General elephant health

Current weight, and weight trends over three to five years including body condition score
Physical examination
Blood work: Complete blood count (CBC), serum chemistry and blood smear
Urinalysis
Acute phase proteins/protein electrophoresis
Fecal analysis for parasites and fecal culture for enteric pathogens
Banking of serum at -80°C for future diagnostics

5. Mtb diagnostic test results

Trunk wash results over 5 years
Other samples submitted for Mtb culture such as semen, milk, feces, biopsies
Serological test results

B. Testing for Mtb in Elephants: Ante mortem and Postmortem Diagnostic Options

Diagnostic tests for Mtb can be applied to elephants ante mortem or postmortem. Ante mortem testing can be done for surveillance, when there is concern or confirmation of disease, or for regulatory purposes, i.e., for moving elephants across state lines.

Two general groups of tests are available: **Direct tests** identify the actual Mtb organism. **Indirect tests** look for exposure to the organism. Culture is the only definitive means of diagnosing infections, although negative results cannot be construed as definitively being equivalent to absence of infection. Tests other than culture can be used when agreed to by parties of origin and destination, but should not be used as sole test for regulatory purposes.

General comments on testing options follow.

Ante mortem direct tests:

Culture: the gold standard for diagnoses of Mtb complex infections in live elephants is culture of trunk wash, TW samples. A positive culture result indicates an elephant is infected but a negative result does not rule out infection. There is the potential for intermittent or low level shedding of bacilli. (Note: a description of the trunk wash technique can be found in Appendix 2). Where available (rare) pulmonary fluid wash or biopsies collected via endoscopy are likely superior to trunk washes due to reduced contamination and the opportunity to collect samples likely closer to or at the site of lesions. (Hildebrandt 2016)

Additional biological specimens that are appropriate for mycobacterial culture, where warranted for a specific instance:

- mucus
- vaginal fluid
- semen
- tissue biopsy
- lung/airway lavage fluid
- esophageal cardia wash
- urine
- feces
- milk

Submission of specimens to laboratories with extensive experience with mycobacterial culture expertise especially with trunk wash samples that commonly are heavily contaminated with normal upper airway microflora (See Appendix 4 for contact information). Duplicate or additional TW samples if collected can be stored in a -30C freezer. The time to obtain culture results is approximately eight weeks.

Polymerase Chain Reaction (PCR): Currently a real time Polymerase Chain Reaction, (RT-qPCR) test on trunk wash fluid is being validated alongside TW culture at the USDA's National Veterinary Services lab in Ames, IA. (Backues 2015) The organism-based test combination of TW culture and qPCR at this lab is showing great promise to significantly improve the sensitivity of the standard TW series as a screening tool for Mtb infection, surveillance, diagnosis, and response to treatment. Note though, that qPCR cannot distinguish live from dead organisms and can detect DNA fragments of organisms. qPCR results are usually returned within a few days. (Magnuson 2017).

Acid-fast staining: acid-fast staining of specimens is appropriate as a fast initial screen, but is not specific for Mtb complex.

Ante mortem indirect tests

Serology: Useful when used in combination with direct testing methods as a screening tool or for monitoring response to treatment. False positives and false negatives are possible and the accuracy of these tests has not been rigorously established. Many elephants are reactive on serological test(s) but are TW negative. Co-editor has treated 2 elephants that were TW positive but serological negative.

Immune response assays (IGRAs); not yet validated for elephants or commercially available (Landolfi et al 2014)

Tuberculin skin testing (PPD) should not be used for Mtb complex infection testing in elephants. (Lewerin, 2005)

Post mortem direct tests

Deceased elephants should receive a full necropsy, and tissue specimens should be collected in duplicate with one set placed in formalin and the other frozen at -80°C. If suspicious lesions such as granulomas, evidence of pneumonia, or enlarged lymph node are evident, or if there is epidemiological or histological evidence suggests of Mtb complex infection, multiple tissue specimens should be submitted for culture, PCR and histological exam. Standard tissues used for tuberculosis diagnosis include but are not confined to:
trunk
trachea
lung
lymph nodes (bronchial, abdominal, cervical, etc), especially if abscessed or enlarged.
stomach wall
salivary glands
small intestine
lower esophagus (cardia)
feces
granulomas - although such lesions should NOT be considered pathognomonic for Mtb complex they are highly associated with TB disease. (Lacasse 2007). Do not assume all granulomas are Mtb, there are other disease that may cause such lesions and other Mtb complex organisms can have similar histologic appearance, verify with culture, and PCR.

A thorough review of the details and logistics of an elephant necropsy can be found in the, **Elephant TAG/SSP Research and Necropsy Protocol of the AZA, June 2017**. This document can be found at **AAZV.org** under SSP necropsy protocols or **AZA.org**
www.aazv.org/resource/resmgr/Protocols/ELEPHANT_NEC_PROT_Jun_2017.pdf
Additional details about the tests below can be found in the ante mortem test section

Culture: Culture samples should NOT be placed in formalin, can be shipped overnight to an appropriate laboratory on a cold pack with appropriate biohazard documentation. Samples can also be frozen until shipping can be arranged. Samples should be double bagged in zippered plastic bags

PCR: PCR samples can be placed in formalin. Jars should be sealed with a wax sealant tape such as 'Parafilm' around the lid, then double bagged in zippered plastic bags

Immunohistochemistry: not routinely performed for diagnosis of Mtb complex in elephants.

Table I: Diagnostic Tests for *Mycobacterium tuberculosis* in Elephants

Test	Type of test	Samples needed	Interpretation of results	Test advantages	Test disadvantages	Availability	Comments
Culture	Direct	Trunk washes most common Other body fluids may be submitted for culture (semen, vaginal secretion, ocular secretion, mucus, lung lavage samples)[34] Post mortem samples either fresh or frozen tissue, including suspicious lesions such as granulomas or caseated lymph nodes, plus lung, trachea, thoracic lymph nodes, salivary gland, and lower esophagus sections. Body fluids as described above.	A positive result from a living elephant indicates active infection and shedding at the time of sampling. A positive result from a necropsy sample indicates infection at the time of death, but not whether the animal was shedding or if the infection were latent. A negative result from a living elephant indicates there wasno Mtb present in the sample tested, elephant was not shedding at the time of sampling. A negative result from a necropsy lesion suggests either that the infection was resolved (only dead organisms present) or that the submitted lesion was not Mtb. qPCR should be performed to confirm presence of Mtb organisms.	The only test that confirms active infection. Test is 100% specific. TW are non-invasive Inexpensive Culture allows for whole genome sequencing and epidemiological investigation	False negatives in live animals due to technique, intermittent shedding, lesion size and location, and activity. Test has poor sensitivity. Slow turnaround for results (10 days to 8 weeks) TW require elephant and staff to be trained for sampling. Lung lavage requires extremely specialized endoscopic equipment plus sedation	Available from laboratories approved for mycobacterial testing and ideally with experience culturing elephant samples since TW are often heavily contaminated and special techniques are needed for processing.	Culture is the gold standard for diagnosis of Mtb in elephants. Trunk wash cultures may also grow nontubercular mycobacteria (NTM) Although two cases of pulmonary disease in elephants have been associated with M. szulgai,[3, 4] NTM grown in a trunkwash do not raise zoonotic concerns or necessitate treatment
Real-time PCR (qPCR):	Direct	Trunk washes from living elephant, other body fluids and mucus. Postmortem samples as described above	A positive result indicates Mtb organisms within the sample, but does not prove active infection since qPCR only confirms DNA presence of an organism, but not its viability. A negative result indicates no Mtb DNA was	Potentially highly specific Can be done using same samples sent for culture Can be performed on formalin-fixed tissue	Testing still in progress, thus sensitivity and specificity are not yet known. For TW, elephant and staff must be trained for sampling	Only one laboratory available in the US (NVSL) Cost is less than $50 US No charge if TW is submitted while validation study is ongoing	Potentially high specificity and sensitivity when done in combination with TW culture particularly with new techniques being developed to enhance the

			present in the samples tested				sensitivity of PCR for TW
Acid fast staining	Direct	Fluid or mucus smear or histopathology	A positive result indicates the presence of organisms with cell walls that resist decolorization with acid or alcohols after Ziehl-Neelsen staining. A negative result indicates that no acid fast organism was present in that particular sample	Very rapid time for results Inexpensive	Very low sensitivity and specificity. Many non-Mtb organisms are acid fast. Many false negatives	Readily available. May be done in-house or by any clinical pathology laboratory	
Serology (e.g. DPP -- Dual Plate Pathway), MAPIA -- Multiple Antigen Print Immunoassay)	Indirect	Serum	A positive result indicates exposure to Mtb complex but does not confirm shedding or active infection. Positive results also have occurred with some tests with M szulgai, an NTM. A treated animal may remain positive for unspecified amount of time after treatment. A negative result indicates either no exposure to Mtb complex or that exposure was too recent for seroconversion, or that elephant did not seroconvert despite exposure (due to low dose of organisms, route of exposure, etc.)	Has potential use as a screening test Rapid turnaround	Elephant must be trained for blood draw. Incompletely documented sensitivity and specificity and not validated in living elephant populations. Mtb, does not stimulate a strong antibody response Cross reactivity with atypical mycobacteria. Poor repeatability with some tests Not recommended for regulatory purposes for reasons listed above; but often used for this purpose.	Availability of some tests has been problematic . Costs highly variable ranging from less than $20 US to $500 US per test. In other species, animal age and health and sample quality affects results[12] which appears to be the case with elephants as well.	Tests use a chromogenic reaction to identify serum antibodies to specific mycobacterial antigens. The degree of color change cannot be correlated with antibody titers at this time. Time course of Mtb antibody production and duration in elephants is unknown.
Cytokine stimulation assays	Indirect	Whole blood	A positive result indicates exposure to Mtb and a functioning innate immune system, specifically cell mediated immunity (CMI) A negative result indicates no exposure to Mtb or a	CMI response is likely more relevant for Mtb diagnosis than evaluating the acquired immune response, thus potentially more sensitive and specific than serology	Validation still underway.	Not available. Experimental only. Some availability in some EU countries	

			nonfunctioning immune system				
Tuberculin Skin Test	Indirect	N/A	Does not work in elephants		Results do not correlate with TB status[10]		Should not be used in elephants. Anecdotal reports that skin test can booster the antibody response in Mtb-infected elephants
Elephant gamma interferon (IFN-γ) test	Indirect	Whole blood	A positive result indicates exposure to Mtb and a functioning immune system A negative result indicates no exposure to Mtb or a nonfunctioning immune system		Validation still underway.	Minimal availability Some availability in Europe	

C. Mtb Risk Categories for Elephants

Elephants are placed into one of three groups depending on their risk of being positive for Mtb. Testing requirements for elephants vary according to what risk group they belong. Note that these groups are determined by two factors: herd history and a positive trunk wash culture, but not by serological test results. The clinical veterinarian can decide to increase the amount and type testing for any elephant based on their concerns and experience. *Caveat: The 'in validation' qPCR on TW samples currently ongoing at NVSL should increase the sensitivity of the standard TW. A positive qPCR test could be a false positive but should be handled as if the sample was Mtb positive while awaiting culture confirmation of the result.*

Category A elephants:

Risk of infection with Mtb complex: Low

Known exposure to an Mtb culture-positive animal: None within the past five years.

Test history: Negative for past five years on annual triple TW testing by culture.

Recommended testing: Routine. Triple TW culture technique done minimally annually.

Travel restrictions: None.

Category B elephants:

Risk of infection with Mtb complex: Moderate

Known exposure to an Mtb culture-positive animal: Exposure to an Mtb culture-positive has occurred within the past five years, i.e., a herd mate was a confirmed positive animal.

Test history: Consistently negative by annual triple TW testing by culture

Recommended testing: Increased. Triple TW cultures should be performed quarterly, every three months for minimum of two years. If no positive results, triple TW cultures may be reduced at the recommendation of the clinical veterinarian but increased surveillance beyond annual testing is recommended for a five-year period. (EX: TW frequency at 4, 3 or 2 x a year) If all tests remain negative after 5-years, these elephants return to Category A status, and only need triple TW cultures once a year.

Travel restrictions: None

Category C elephants:

Risk of infection with Mtb complex: These elephants are positive on TW cultures or culture of other body fluid.

Known exposure to an Mtb culture-positive animal: Animals with whom this elephant has been in contact become Category B elephants. Trace back to see what other elephants and other animals should be performed to determine what other transmission routes were possible. Human exposure to Category C elephants should be handled with the cooperation of local and state public health officials. See **THE TRUNKWASH POSITIVE ELEPHANT: FIRST STEPS,** following Table 2.

Test history: Positive on a single or multiple TW cultures or cultures of other fluids

Recommended testing: Increased on several levels. See next section: Next steps following identification of an Mtb culture positive elephant (Class C elephant).

Travel restrictions: Travel permitted only for specific medical reasons

Table 2, below, summarizes the categories.

Table 2: Risk categories of elephants with regard to Mtb

Category	Risk of infection with Mtb	Known exposure to Mtb	TW results	Frequency of testing	Travel restrictions	Status changes
A	Low	None within past 5 years	Negative for past 5 years on all cultures	Triple TW done once/yr. Herd surveillance acceptable in some cases	None	None
B	Moderate	Exposure to a culture positive herdmate (a category C elephant) or human caretaker has occurred within the past 5 years	Negative for past 5 years on all cultures	Increase triple TW frequency to 4 times/yr for 2 years. Increased TW frequency recommended for full 5 years.	None	After 5 years of increased TW, if all are negative, elephant reverts to Category A
C	Confirmed infected by culture	Trace backs are needed to determine animals with whom this elephant has had contact	Positive on a single or multiple TW	Increased on many levels. See pages 17, and treatment sections	Travel only permitted for medical reasons.	Herdmates of a class C elephant become Class B elephants.

*TW = Trunkwash

THE TRUNKWASH POSITIVE ELEPHANT: FIRST STEPS

After an elephant tests positive for Mtb via TW culture, the diagnostic testing laboratory will contact the attending veterinarian. Once a positive culture is received, the elephant is considered infected with Mtb, and the following notifications are necessary and even before confirmation of results:

Although a positive qPCR result may represent a false positive for active disease, the initiation of the following steps should be undertaken while waiting for confirmatory culture results.

A. Notifications of an Mtb-culture positive elephant must include:

1. Regulatory personnel such as:
   - State Veterinarian
   - State Public Health Veterinarian
   - local public health officials
   - USDA VS Inspector veterinarian for the facility

2. Facility personnel including:
   - All staff working with the elephant including barn staff, veterinary staff and volunteers
   - Upper management & legal teams
   - Public relations, marketing, and communications teams
   - Human resources
   - Safety departments

3. Notification of other facilities where the Mtb culture-positive elephant has been, if appropriate.

Discussions regarding safe elephant handling, use of personal protective equipment (PPE) such as N-95 respirators, and barn cleaning protocols to prevent zoonotic transmission or spread to other elephants in the herd should occur in collaboration with regulatory and facility personnel. See Human Health Considerations starting page 33.

B. Confirmation of the positive TW culture result.

- If TW samples have been banked, submit the duplicate sample to the lab for culture
- Submit a new TW sample for culture.

C. Additional information needed from the diagnostic laboratories:

1. Request additional identification of the Mtb isolate using whole gene sequencing from the diagnostic lab. There is a moderate fee for this service but older tests such as spoligotyping yield minimal information as to the genetic relatedness of Mtb strains.

2. Test for antimicrobial susceptibility using validated CLSI references.

Note: if the laboratory is not able to perform these tests, it should ship the isolate to National Veterinary Services Laboratory (NVSL) (contact information can be found in Appendix 4.)

3. Identify laboratory that can measure serum drug samples, and confirm testing and handling protocols (See Appendix 5)

D. Purchase and testing of antitubercular drugs.
1. Costs and sources for large amounts may vary; comparison shopping is recommended
2. Purchase drugs only from a licensed pharmacy and certified compounder
3. Permission from the FDA may be needed to import bulk drugs
4. Priority drugs to source are first-line drugs, Isoniazid (INH) and Rifampin (RIF).
5. New batches of bulk drugs should be tested for purity and drug activity concentrations. Samples can be submitted to the Infectious Disease Pharmacokinetic Laboratory at the University of Florida (Contact information is in Appendix 5).

E. Sourcing Medications:
1. The use of bulk drugs may be a viable alternative to the use of FDA approved TB products due to elephant size, the length of treatment and the ability to properly formulate/compound for administration to elephants.
2. Some distributers of FDA approved products in the past have restricted the dosage units that can be purchased which means not enough could be purchased for use in elephants.
3. Bulk drugs contain an active pharmaceutical ingredient (API) typically used for human drug compounding
4. In contrast, FDA approved TB products come in fixed dosage forms for human use.
5. The FDA recognizes that there are specific circumstances when an animal drug can be compounded from bulk drug substances.
6. The FDA has the authority to regulate compounding for animal use and offers guidance in purchasing active pharmaceutical ingredients (bulk drug) for treatment options.
7. The FDA has released a draft: "Guidance for Industry (GFI) #230 Compounding Animal Drugs from Bulk Drug Substances."

GFI #230 outlines the current FDA stance for these circumstances.

GFI#230 also documents the necessary items that must be included on a prescription for a pharmacy to obtain bulk drug to be dispensed or compounded.

A link to that draft can be found at:

http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM446862.pdf

F. Purchasing Drugs
1. Shortages resulting in delay of delivery for several weeks have occurred.
2. Always verify drug availability with a pharmacy prior to purchase and discuss duration of treatment (total amount of drug needed) with that pharmacy.
3. Bulk drugs may be purchased by **direct importation** from a manufacturer or via **purchase from a licensed pharmacy**.

If directly importing bulk drugs from a manufacturer:

Verify that the importing entity is registered with the FDA.

Check state rules regarding the purchase and distribution of bulk drugs.

Check if state rules require the importer to have a license equivalent to a pharmacy wholesale license or distribution license prior to purchase or sale of bulk chemicals (aka drugs).

4. If purchasing directly from a licensed FDA-approved facility, a certificate of analysis (COA) from the original manufacturer will accompany the product. The COA documents the potency of the bulk drug.

If purchasing bulk drugs from a pharmacy:

Check first with local compounding pharmacies which may have Mtb drugs available at reasonable prices.

Larger veterinary pharmaceutical compounding pharmacies have routinely stocked Mtb-related bulk drugs for the last several years.

When purchasing from a pharmacy, generally a COA does not come with the prescription but can be requested from a dispensing pharmacy.

G. Concerns with bulk drug use

1. Potency of bulk drugs has been a problem.
- Loss of potency of bulk drugs, can occur for multiple reasons.
- Due to the possibility of loss of potency, samples of the bulk drug can be analyzed via a potency assay.
- A few facilities that will perform potency analysis are listed here, but many others are available.

Analytical Research Laboratories (ARL), 840 Research Parkway, Ste. 546, Oklahoma City, OK 73104, 800-393-1595

Compounder's International Analytical Laboratory (CIAL), 680 Atchison Way, Suite 100, Castle Rock, CO 80109, 800-788-9922

Front Range Labs, 3985 S Lincoln, Loveland, Co 90537, 970-593-0171

Professional Compounding Centers of America (PCCA), 9901 South Wilcrest Drive, Houston, TX 77099, 800-331-2498

TREATMENT OF MTB CULTURE POSITIVE ELEPHANTS

A. Definition of Epidemiological Terms: See Appendix 6.

B. Goals of treatment:

1. Stop shedding of live Mtb organisms to protect herdmates and humans.
2. Prevent elephant from becoming ill from treatment.
3. Obtain appropriate serum/plasma concentrations of antitubercular drugs in the elephant.
4. Monitor TW cultures intensively throughout treatment. See Category C elephant requirements.
5. Treat for an adequate period of time, typically one year.

Caveats: Doses, frequency, and duration of treatment remain empirical in elephants.

C. Overview of anti-tubercular drugs regimen:

1. Mtb in humans is typically treated with a four-drug regimen for 6 to 12 months.
2. Elephant protocols have used human protocols as a starting point.
3. Drugs typically used are isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (ETH).
4. INH and RIF (or a fluoroquinolone in place of RIF when compliance is an issue) are recommended for all treatment regimens.
5. Pharmacokinetic (PK) 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 concentrations.
6. The PK and pharmacodynamics (PD) of these drugs in elephants are quite different compared to humans.
7. A fine balance exists between giving an amount of drug that does not cause adverse effects and one that does not select for resistant organisms. Optimization of drug doses must be tailored to each individual elephant's tolerance to medications. It is not uncommon to alter the dose or the frequency at which a medication is given during the treatment period to accommodate the elephant's tolerance to medications, and changes in appetite and attitude.
8. Elephants need be trained to take drugs consistently, regardless of route. Treatment will be most effective if training is well established and routine before the elephant has a medical need to take medications.

Routes of drug administration in elephants are described in Table 4, below.

Selected references: (Maslow et al 2005a & b: Zhu et al 2005, Peloquin et al 2006)

D. Characteristics of Drugs Used to Treat Mtb in Elephants

1. Isoniazid, INH

INH should be used in all elephant protocols unless the elephant is intolerant of the medication or the mycobacterial isolate is found to be insensitive to it.

INH is responsible for the rapid killing of actively dividing Mtb organisms.

2. Pyrazinamide, PZA

Used in conjunction with INH to prevent the development of INH resistance.

INH and PZA should always be used initially for Mtb treatment in elephants and can be effectively administered together rectally.

3. Rifampin, RIF

RIF kills latent or inactive Mtb organisms and resolves cavitary lesions.

A drawback for RIF's use in elephants is that it is only absorbed orally. Every effort should be made to train elephants to consistently accept oral medications and all attempts exhausted to get consistent long term RIF administration during treatment. In humans the presence of this drug in a TB treatment regimen is highly correlated with lack of relapse infection. If consistent RIF administration is not possible or not reliable then another drug with similar properties should be selected. See Appendix 8 for a novel method of making oral RIF capsules that masks taste.

4. Fluroquinolones:

Levofloxacin (LEVO) is an approved second line drug for the treatment of human TB infections and can be used in place of or in combination with RIF in an attempt to treat active and potentially latent populations of Mtb organisms and prevent relapse of infection. Their efficacy for resolving infections is not well documented compared to what is known about RIF's association with success. Enrofloxacin has been used in elephant treatment regimens but its metabolite, ciprofloxacin is not considered an anti-tuberculosis drug in the treatment of human TB infections and the organism has been shown to develop rapid resistance to ciprofloxacin. (Gumbo 2005) Until more is learned about the efficacy of Enrofloxacin it is advisable to use Levofloxacin in the treatment of elephant infections.

LEVO can be given orally or rectally

Rectal administration of LEVO typically requires 4-5 x the oral dose to achieve effective blood concentrations.

When administered rectally with other medications such as INH and PZA there has been lower blood concentrations achieved of LEVO. (M. Finnegan personal communication and Co-editor personal experience)

Selected references: (Filippini 2015), (Spigelman 2007). (Leibert 2010), (Garcia-Tapia 2004)(Gumbo 2005)

5. Ethambutol, ETH

ETH is similar to PZA in being symbiotic with the other drugs

ETH is important in preventing failure of treatment due to resistance.

ETH must be administered orally to be absorbed and therefore has similar issues with compliance as does RIF.

See Appendix 8 for a recipe/method successfully used by zoo to orally medicate an Mtb infected elephant.

Table 3: Antitubercular drug characteristics and usage

DRUG	MECHANISM OF ACTION	REASON FOR USE	ROUTE	DOSE	USE & DOSE	COMMENTS
Isoniazid (INH)	Inhibits synthesis of mycolic acids in the bacterial cell wall.	Rapid killing of actively dividing Mtb organisms.	Oral or rectal	2-5 mg/kg	Initiation phase at 2-3 mg/kg followed by Continuation phase at 5-7 mg/kg OR Combination protocol at 5-7 mg/kg	INH should be used in all Mtb treatment protocols unless elephant is intolerant of it
Rifampin (RIF)	Inhibits DNA-dependent RNA polymerase activity in the bacteria.	Kills latent or inactive Mtb organisms and resolves cavitary lesions.	Oral only	10 mg/kg	Continuation phase at 10 mg/kg OR Combination protocol at 10mg/kg Combination protocol at 10 mg/kg	If the elephant is not trained for consistent RIF oral ingestion, substitute ENRO or LEVO.
Ethambutol (ETH)	Diffuses into actively growing Mycobacteria and inhibits cell wall biosythesis. This leads to increased cell wall permeability and bacterial death.	Symbiotic with the other drugsImportant in preventing failure of treatment due to resistance.	Oral only	15 mg/kg	Initiation phase, Continuation phase OR throughout Combination Therapy at 15 mg/kg	Do not use alone; must be used in conjunction with other drugs.
Pyrazinamide (PZA)*	After metabolism into pyrazinoic acid, it interferes with Mycobacteria's ability to synthesize the fatty acids it requires for growth and replication.	Used in conjunction with INH to prevent resistance	Oral or rectal	20 mg/kg	Initiation phase in combination with INH at 20 mg/kg Or Combination Protocol 20-30 mg/kg	Do not use alone; must be used in conjunction with other drugs.
Levofloxacin (LEVO)*	fluoroquinolones inhibit bacterial topoisomerase and DNA gyrase, enzymes needed for bacterial DNA replication, transcription, repair	Treats latent populations of Mtb organisms and to prevent relapse.	Oral or rectal	5 mg/kg Oral dose only	Oral at 5 mg/kg OR Rectal at 15-25 mg/kg*	Can be used with or as a substitute for RIF of RIF is not accepted by elephant.

*When administered rectally with other anti-tubercular drugs such as INH & PZA lower blood concentrations may be seen. Important to check blood concentrations and adjust dose accordingly

Table 4: Routes of drug administration in elephants

Route	Technique	Advantages	Disadvantages	Drugs absorbed by this route	Comments
Oral*	Animal asked to open mouth. Entire dose is administered either as tablets, capsules or mixed in suspension & delivered by syringe. Some elephants can be trained for a bite block	Not painful	Animals may refuse or spit out medication, or hold meds in mouth for a long time without swallowing. Hepatotoxic drugs may show greater toxicity due to first pass effect which is avoided if given rectally	PZA, LEVO, RIF, ETH, INH.	Requires training. All elephants should be trained to accept oral medications before a situation arises that requires routine consistent medication
Rectal	Drug is dissolved or suspended in water and administered into the rectum via dosing syringe and tubing. Some manure must be gently evacuated from the rectum prior to administration	Efficient administration of drugs. Eliminate first pass effect which may reduce toxicity and allow lower total dose to achieve same serum levels.	Can cause irritation of the rectum. Not all drugs can be given by this route.	PZA, INH, LEVO**	Requires training. Elephants should be trained to accept rectal exam, enema and rectally administered medications as part of basic animal care.
Injectable	Drugs are given intramuscularly with a needle and syringe	Easy to confirm that entire dose was given.	Painful. Causes muscle damage and can be associated with a risk of abscess formation.	ENRO, Efficacy unknown and not considered a 2nd line drug in TB treatment	Not recommended for elephants long term.

*See Appendix 8 for a novel approach to oral capsule manufacture and acceptance.
**See previous statements on lower blood absorption of some medications when given together.

TREATMENT PROTOCOLS FOR CULTURE POSITIVE ELEPHANTS

(Note**:** This section was adapted from the *ATS/CDC/IDS Practical Guidelines for Drug Susceptible TB-CID 2016:63*)
https://www.cdc.gov/tb/publications/guidelines/pdf/clin-infect-dis.-2016-nahid-cid_ciw376.pdf

A. Overview of Treatment

1. Treatment recommendations for elephants resemble human protocols in utilizing a two phase protocol, but some veterinarians have used only the initiation phase treatment regimen throughout the entire course of treatment without adverse effects:

a. The first is the Initiation Phase, characterized by frequent administration of drugs.
b. The second is a Continuation Phase in which the doses of each drug are typically higher but are administered less frequently.

2. A Single Phase Combination Therapy protocol is possible for elephants that become very sick during the intensive Initiation phase and consists of only a Continuation phase like schedule.

a. This protocol should last longer, ~ 18-24 months.
b. Drugs are given intermittently (3 to 4 times/week), but at higher doses.
c. In humans, this schedule has been shown to be less effective than a two-phase protocol.
d. There is no data on whether this is also true in elephants.
e. Its use should only be considered in elephants that cannot tolerate the more frequent dosing regimens.

3. If at any point during the Single Phase Combination Therapy Protocol or during the Continuation Phase of the Two-Phase Protocol, a positive TW by culture occurs, the sample should receive full whole genome sequencing, WGS and repeat bacterial susceptibility testing. See page 29 for a discussion of treatment failures.

4. To stop shedding, increased frequency of dosing during the first few months of treatment (initiation phase) is likely applicable to elephants.

5. Some drugs have post-antibiotic effects, i.e., they continue to function even after their serum concentrations are decreased. This enables an intermittent dosing schedule to be effective, this is the ideal behind how the continuation phase works.

6. To try to stop bacterial shedding as quickly as possible**,** start rectal administration of INH with PZA as soon as possible rather than wait for susceptibility results.

Selected references: (Backues 2015) (Simpson 2017)

B. Two Phase Protocol: Initiation phase and Continuation Phase

1. Initiation phase

- Goals:
  - Rapidly decrease large living Mtb bacterial populations without creating resistance
  - Stop detectable shedding
- Schedule: Administer 3-4 drugs concurrently during the first ~ 3 months of treatment
  - Give drugs either every day, or 5 days a week with 2 non-consecutive days off
  - Example: Drugs administered Sunday, Monday, Tuesday, Thursday & Friday
    No drugs are given on Wednesday or Saturday
- Monitoring: Perform at least one TW per week throughout this phase to monitor shedding.

-Shedding should be verified to have ceased during this phase as one of the signs of effective therapy.

-Some veterinarians have continued this type of regimen through an entire 12- month course of treatment, but this is dependent upon elephant tolerance to medications. There is currently no data as to whether a single intensive treatment or changing to a continuation phase are equally effective. (M. Finnegan personal communication)

2. Continuation phase

- Goals:
  - Continued killing of Mycobacterial organisms, including inactive or latent bacteria.
- Schedule: Administer 2-3 drugs concurrently for the remainder of the approximately 12- month treatment regimen
  - Drugs are given on intermittent schedule but doses are increased.
  - Administration can be decreased to minimum of 3 treatments a week.
- Monitoring
  - Monitoring for shedding must continue throughout treatment
  - Perform a minimum of one triple TW series each month throughout this phase

C. Single Phase Combination Therapy Protocol

- Goals: Eradication of Mtb in situations where elephants cannot tolerate the intensive Initiation phase of the two phase drug protocol.
- Schedule: Administer 3 drugs concurrently for the entire treatment regimen which is should be increased to a minimum of 18 months.
  - Give drugs on an intermittent schedule of 3 to 4 times per week.
  - Use doses that are 2x the doses used when an Initiation phase is being utilized.
- Monitoring
  - Monitoring for shedding must continue throughout treatment
  - Perform a minimum of one triple TW series each month throughout this phase

Table 5: Example of a Two-Phase Mtb Treatment Protocol for Mtb in an Asian Elephant.[a]

Treatment phase	Drug	Frequency	Notes
Initiation	INH 2-5 mg rectally plus PZA 20 mg/kg rectally plus RIF 10 mg/kg orally and LEVO, 15 mg/kg rectally (can be used if RIF is not possible) ETH, 15mg/kg orally	5 days/week	INH & PZA should be started as soon as possible and even before other drugs or susceptibilities are completed. Combination of 3-4 drugs should continue ~ 12 weeks. Cessation of detectable shedding of Mtb organisms by Culture is sign of effective treatment. Elephant's tolerance of all these medications is on an individual basis. The elephant should receive weekly TW testing by combined culture and qPCR (if available) throughout this phase. Duration of the initiation phase may need to be extended if there are compliance issues or if shedding continues (as determined by TW monitoring.) LEVO can used in conjunction with RIF based on susceptibilities and the elephant's compliance with taking oral medications. When RIF is not possible to be administered LEVO may be used but RIF is preferred.
Continuation	INH 5 mg/kg rectally plus RIF, 10 mg/kg orally and/or LEVO, 15 mg/kg rectally	3 days/week	Combination of at least 2 drugs but 3 maybe more desirable Continue for duration of treatment regimen

[a] Sample protocol only. Although elephants have been treated with similar protocol, other elephants may not tolerate these medications or doses, or may not respond to treatment.

MONITORING DURING TREATMENT FOR MTB

A. Increased frequency of trunk washes

1. Regardless of the treatment schedule, an increased number of TW should be performed throughout to determine if shedding has stopped.
2. If using the two-phase protocol, submit a single trunk wash (TW) or triple TW series for culture each week throughout the Initiation Phase. Bacterial shedding should cease during the Initiation Phase and this phase can be increased in duration if tolerated.
3. Submit a triple TW series once a month and throughout Continuation Phase until treatment is completed.
4. If using the single-phase Combination protocol, triple TW series weekly should continue until detectable shedding has ceased, then reducing to monthly TW monitoring sometime after cessation is verified.
5. If any TW are culture positive during treatment, recheck sensitivity of the organism and repeat whole genome sequencing, WGS again to assess if a new isolate is present. The following options can be considered:
    - Increasing the amount of INH per dose
    - Adding in a fifth drug
    - Swapping one drug for another comparable drug, while keeping INH in the mix.
6. Some elephants shed infrequently, and true cessation may be difficult to discern.

B. Health assessments of elephant receiving treatment

1. Routine CBC/Chem/UA and blood smear should be performed monthly
2. The elephant should be assessed daily for its tolerance to the drugs.
3. If the elephant shows obvious signs of illness during treatment, additional diagnostics may be needed. See page 31 managing adverse effects in elephants.
4. A body weight should be measured quarterly.
4. The use of serology to monitor post-treatment recovery is un-validated, but some serologically reactive animals may eventually become non-reactive.

C. Monitoring Herdmates

All herdmates of the infected elephant become Class B elephants and should receive triple TW testing every 3 months.

D. Measuring drug concentrations

1. Drug concentrations are used to determine if the elephant is reaching a blood concentration of drug known to be effective against Mtb organisms in other species.
2. Therapeutic drug concentrations for humans are often not attainable in elephants so comparing the drug concentration reached with the Mtb isolates susceptibility, MIC is often used to assess treatment concentrations.
3. Some evidence shows that the amount of drug a particular elephant needs can have high individual variability.
4. Drug concentrations can be measured in serum or plasma. Speak with the laboratory running the samples to find out which is preferred.
5. The goal is to create a PK curve to determine the animal's drug concentration is at $\mathbf{T_{max}}$. Table 6 shows typical T_{max} times for different drugs.

6. T_{max} is the time after administration of a drug when the maximum plasma concentration is reached.
7. Not all elephants will reach an appropriate drug level within the expected time. Some will take longer, and some will never reach an appropriate serum level of a specific drug at the dose being used.
8. Some drugs may inhibit the rectal absorption of other drugs (eg, rectal levo suspension and INH) so if adding a new drug to the protocol repeat PK trials on all of the drugs.
9. If the elephant is not reaching appropriate drug levels, concerns regarding measuring or understanding drug levels should be discussed with the laboratory. Consider purity testing and examine sample handling techniques (PK serum/plasma samples) The drug may need increased or effective therapy may be based on the isolates susceptibilities and cessation of shedding.
10. Plan for measuring drug concentrations once elephant is consistently taking medications early in treatment regimen, within first 14-45 days. Compare concentrations with MIC of organism as well as TW shedding results, and appropriate drug concentration listed for humans. Drug concentration monitoring should be repeated ~ ½ way through treatment or anytime doses or administration rates are changed. Consistent results with same drug and dose are not always seen.
Selected References: (Alsultan 2014)(Egelund 2015)(Wiedner & Hunter 2013)

E. Specifics of measuring drug concentrations.
1. Blood is collected at specific timed intervals after rectal or oral administration of a particular antibiotic, starting at Time Zero (T_0; i.e., immediately before drug administration)
2. After T_0, additional blood samples are collected every 15 or 30 minutes until one hour past T_{max}. Each blood sample is put into its own vacuum tube.
3. Use lithium heparin vacuum tubes for plasma collection.
4. Use red top vacuum tubes for serum collection (but not serum separation tube –red/grey tops).
5. If collecting serum, allow blood to clot in the red-top vacuum tube at room temperature, then spin down the tube in a centrifuge for 5 minutes at 3500 RPM.
6. Plasma does not require waiting for clotting to occur before centrifugation, so the lithium heparin tube can be spun as soon as it is filled.
7. After centrifugation, use a disposable pipette to transfer the serum or plasma into a freezer proof plastic tube.
8. After labeling the tube with the animal's name, whether the tube contains serum or plasma, the date, and how long the contents were collected after antibiotic administration, place the tube in a standard freezer if samples are going to be sent out for exam in a short period of time. Long term storage of samples can be done in a -80C freezer.
9. Discuss shipping protocols with the laboratory prior to packaging.
10. Example: Determining drug serum concentrations at T_{max} for rectally administered INH
    Look at Table 6: T_{max} for INH is 3 hours
    You can take first blood sample at T0 before elephant is medicated.
    Administer full dose of INH rectally.
    Take blood every 15 minutes for first hour
    Then take blood at 30 minute intervals until 3 hours past T0
    Take last sample at Hour 4. There will be a total of 9 samples.
    Plasma or serum should be frozen soon after collection since some of the drugs are labile and photosensitive in the blood.

Plasma samples can be submitted to Dr. Charles Peloquin at peloquinlab@cop.ufl.edu. See Appendix 5 for contact information.

Table 6: Time to T_{max} for antibiotics used against Mtb infection in elephants

Drug	Route	Usual time to 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
Levofloxacin	Oral and Rectal	Approximately 6-8 hours

*T_{max} = time after administration of a drug when the maximum plasma concentration is reached;

F. Recommended Antibiotic Blood Concentrations for Treatment of Mtb in Elephants

Recent evidence indicates considerable 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 7: Starting drug doses for treatment of elephants with Mtb

DRUG	PHASE OF THERAPY	ROUTE**	DOSE
Isoniazid (INH)	Initiation Phase	Oral or rectal	2-5 mg/kg
Isoniazid (INH)	Continuation Phase or Combination Therapy	Oral or rectal	5-7 mg/kg
Rifampin (RIF)		Oral	10 mg/kg
Ethambutol (ETH)*		Oral	15 mg/kg
Pyrazinamide (PZA)*		Oral or rectal	20 mg/kg
Levofloxacin (LEVO)		Oral	5 mg/kg
LEVO		Rectal	15-25 mg/kg

*ETH and PZA should not be used alone but are adjuncts to drugs such as INH to help prevent development of resistance. PZA has been most commonly used for treatment of elephants due to ability to administer rectally at same time as INH.

** Note that any routes of administration require prior training of the elephant

G. Overview of adverse effects in elephants associated with antitubercular drugs

1. All antitubercular drugs can cause adverse effects.
2. Combining drugs may increase the likelihood of negative effects.

3. Which drug is causing problems is not always clear, and sometimes it is the combination together that creates adverse effects.
4. Serious adverse events are well-documented in humans, domestic animals, and elephants.
5. The adverse signs seen in elephants are more like those seen in domestic animals, rather than those that occur in humans.
6. Some signs may be transient and resolve spontaneously; others may persist
7. Adverse effects can occur in elephants at doses of drugs recommended for humans, suggesting that human serum concentrations can be toxic for elephants.
8. One of the most serious adverse effects with these drugs in humans is liver toxicity, leading to liver failure.
9. In humans and elephants, increased liver enzymes may be seen on bloodwork obtained during treatment. However, in elephants, liver enzymes vary in specificity and are poorly validated.
10. Bile acids, the gold standard for monitoring liver function in humans, do not occur in elephants. Thus, monitoring for hepatoxicity in elephants can be very challenging.
Selected References: (Wiedner and Schmitt 2007), (Wilson 2010)(Simpson 2017).

H. Specific Signs of Drug Intolerance/Adverse effects in Elephants

1. Partial to complete anorexia
2. Depression
3. Hard, scant, black, and or fetid manure
4. Severe blepharospasm
5. Ocular tearing
6. Weakness.
7. Small amounts of frank blood in feces associated with rectal administered drugs
8. Colic/abdominal pain
9. Liver failure
10. Death

I. Managing adverse effects of drugs in elephants

1. If signs are mild such as ocular tearing and blepharospasm, providing analgesia and keeping the elephant out of bright sunlight may be helpful.
2. If signs do not abate or are severe enough to cause significant discomfort or distress, temporary cessation of all drug treatment is warranted.
3. Obtain routine bloodwork (CBC/Chem/blood smear) if adverse signs are noted.
4. Most signs will resolve within 7-10 days of stopping all antibiotics.
5. Supportive care for the sick elephant may include hydration using rectal fluids and analgesics.
6. Restart drugs once the elephant appears to be recovered. Repeat the CBC/Chem/blood smear.
7. If signs recur, consider using the Single Phase Combination Therapy Protocol (see page 27).
8. If signs recur, consultation with an experienced veterinary pharmacologist or zoo veterinarian that has treated elephants with Mtb may be helpful in determining if other drugs or drug protocols may improve the situation.
9. 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. (see Appendix 4 for references).

J. Treatment failures

1. Recurrence of shedding (a positive TW) indicates treatment failure.
2 Treatment failure can occur at any point during or after treatment. Humans are typically monitored for two years after completion of antitubercular treatment.
3. Recurrence (also called relapse) rates in humans using a four-drug approach range from 0 to 27%.
4. Prevalence of treatment failure in elephants is unknown.
5. If weekly TW cultures continue to be positive eight weeks after starting treatment:
   Recheck drug sensitivities
   Confirm potency of medications being administered
   Recheck serum drug concentrations.
6. Reported cases of INH resistant infections have occurred in elephants. If this is noted, consider using a combination of RIF & Fluoroquinolone medications in place of INH based on reported susceptibilities.
7. For resistant infections and/or extreme elephant sensitivity to drugs, few guidelines exist. Thus, 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 veterinary experts to determine how best to proceed. An excellent reference guide for physicians dealing with Drug-Resistant TB in humans may also offer some guidance for veterinarians in this difficult situation.

Curry International TB Center, Drug-Resistant TB, A Survival Guide for Clinicians.3rd Ed
http://www.currytbcenter.ucsf.edu/products/cover-pages/drug-resistant-tuberculosis-survival-guide-clinicians-3rd-edition

K. Recurrence vs Reinfection

1. Re-infection is a new infection with a different isolate and is considered different from recurrence.
2. Distinguishing between recurrence and re-infection requires molecular techniques such as whole genome sequencing to determine if the new isolate is the same strain as the initial one and if it has mutated. (Bryant 2013)

L. Monitoring after treatment is completed.

1. Test by triple TW culture series every 3 months for 2 years.
2. If all TW are culture negative during this period, the elephant reverts to Category B and should be tested as per that protocol: by triple TW technique every 3 months for an additional 2 years, and followed with increased TW monitoring for the remaining 3 years.
3. At this point (2 years), the elephant no longer has travel restrictions.
4. Herdmates continue as Category B elephants for the recommended time.

HUMAN HEALTH CONSIDERATIONS

A. Human-to-human transmission of Mtb

1. Transmission of Mtb between humans is well studied and comparatively well defined. Transmission can occur when Mtb are aerosolized by a person actively shedding the bacteria. 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
2. Indirect contact with a person who is shedding the organism is unlikely to result in transmission. Humidity and light do not affect transmission.
3. 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 are contacted for testing in a TB exposure investigation.
4. Other routes of Mtb exposure in humans have not been documented or are not considered significant.

Selected references: (Heyman 2008)

https://www.cdc.gov/tb/education/corecurr/pdf/chapter2.pdf
WHO Guidelines, Tuberculosis and Air Travel: Guidelines for Prevention and Control, third edition, 2013)

B. Elephant-to-human transmission of Mtb

1. In general, Mtb requires prolonged, close contact with a person or animal that is actively shedding the bacteria into the environment. Transmission of Mtb from elephants to humans is, therefore, more of an occupational health concern than a health concern for the general public.
2. Close, prolonged contact as a significant risk factor for transmission is supported by an investigation that assessed 48 zoo patrons, 20 of whom were present at special elephant events where a culture positive elephant actively aerosolized paint with his trunk. All zoo patrons had <30 minutes of exposure at a distance of $\geq$ 25 feet to this elephant during his presumed infectious period and no patrons evaluated showed evidence of exposure. In contrast, that same study documented skin test conversions in 5/31 zoo employees, all of whom were considered close contacts to this same elephant, and one zoo volunteer, who had greater exposure (1 hour cumulative presence in the elephant barn) than zoo patrons to the same culture positive elephant (Zlot, et al, MMWR 2016). 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.
3. Close prolonged contact such as routine elephant handling, elephant training, or participating in a necropsy on an Mtb-infected elephant can increase occupational risk. (Michalak 1998)
4. Cleaning protocols that utilized high pressure water hoses were implicated in causing humans working close to a barn containing an Mtb-infected elephant to become PPD (skin-test) positive, although they did not develop active infections (Murphree 2011). Thus, husbandry practices should be examined closely.

Selected references: (Zlot 2016), (Davis 2001), (Murphree 2011), (Oh 2002), (Montali 2001), (Mikota and Maslow 2011), (Michalak 1998), (National Tuberculosis Controllers Association 2005), (Vogelnest 2013), (Lecu and Ball 2011).

C. Human-to-elephant transmission of Mtb

1. Initial infections of elephants with Mtb have been hypothesized to come from close contact with infectious humans and some documented evidence exists to support this suspicion. (Payeur 2002). Several elephants in the US originally imported from South East Asia have had Mtb isolates that originate genetically (Lineage-3 genotype) from this part of the world. (S. Robbe-Austerman, K Backues personal communication)
2. Mtb infection has only recently been documented as a disease of wild elephants. (Chandranaik 2017), (Perera 2014))

D. Occupational Health Recommendations

1. Managers and veterinarians of facilities that maintain elephants should consult with that facility's occupation health staff, State Public Health Veterinarian and/or health department tuberculosis staff to develop an occupational health program. Program development activities should include a written infection control plan that covers measures designed to reduce direct and indirect aerosol transmission of Mtb. Such measures may include addressing use of personal protective equipment (PPE), elephant quarantine procedures, disinfection procedures and the ventilation in a facility where elephants are being housed.
2. Facility protocols should assign levels of risk for Mtb exposure based on factors including differing levels of contact staff have with elephants, species of elephant, diagnostic history of the herd and degree of public contact.
3. Protocols should include routine annual tuberculosis screening of employees who work with elephants and testing of new hires. Employees with acid-fast positive sputum smears or suspicious chest films should not work directly with elephants until they have had additional diagnostic testing. The results of this additional testing should inform decisions about an employee's return to work and performing elephant care duties. Human testing for Mtb should be overseen by occupational health and/or tuberculosis control authorities.
4. Managers of elephant facilities should encourage employees who have health concerns to discuss them with a healthcare provider, occupational health staff and/or health department personnel. Managers should also understand that employee medical information is considered private and discuss with health department and/or occupational health personnel how situations associated with an employee for whom Mtb infection is a concern will be handled.
5. Routine education of staff in zoonotic disease prevention should occur, including discussion of clinical symptoms consistent with active human tuberculosis infection.
6. Employees should be trained in the proper use of PPE.

Resources for Occupational Health and Safety Information can be found in Appendix 7.

Appendix 1: References: Most are cited in text but others are included as an overview of current and historical literature on Mtb in both human and veterinary medicine.

1. Alsultan A, CA Peloquin Therapeutic Drug Monitoring in the Treatment of Tuberculosis: An Update. Drugs 2014: 74, Pp839-854.

2. American Thoracic Society, Centers for Disease Control and Prevention, Infectious Diseases Society of America. Treatment of tuberculosis. Morbidity and mortality weekly report 2003;52:1-80.

3. Angkawanish T, Morar D, Kooten Pv, et al. The elephant interferon gamma assay: a contribution to diagnosis of tuberculosis in elephants. Transboundary and emerging diseases 2013;60:53-59.

4. Backues KA, Robbe-Austerman S, Isaza R. Documented cessation of mycobacterial shedding with antibiotic treatment in a *Mycobacteria*-tuberculosis-positive Asian elephant (*Elephas maximus*) by serial culture and direct real-time polymerase chain reaction testing of trunk wash samples Proc AAZV 2015;151-152.

5. Bontekoning I. Tuberculosis detection in the Asian elephant (*Elephas maximus*) population of Thailand. 1. Development of an IFN-gamma assay 2. Evaluation of a multiple antigen iELISA and a commercial rapid test. In. Thailand: Kasetsart University, Bangkok, Chiang Mai University, Chiang Mai Utrecht University, Utrecht; 2009:29 pages.

6. Boyd J. Serum enzymes in the diagnosis of disease in man and animals. Journal of comparative pathology 1988;98:381-404.

7. Blumberg H, Burman W, Chaisson R, et al. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. *Am J Resp Crit Care Med* 2003;167:603.

8. Brock AP, Isaza R, Egelund EF, et al. The pharmacokinetics of a single oral or rectal dose of concurrently administered isoniazid, rifampin, pyrazinamide, and ethambutol in Asian elephants (*Elephas maximus*). Journal of Veterinary Pharmacology and Therapeutics 2014.

9. Bryant JM, et al. Whole-genome Sequencing to Establish Relapse or Re-infection with Mycobacterium tuberculosis: A Retrospective Observational Study. The Lancet 2013 V1, Pp786-792.

10. Chalke HD. The impact of tuberculosis on history, literature and art. Medical history 1962;6:301-318.

11. Chandranaik B, Shivashankar BP, Umashankar KS, et al. *Mycobacterium tuberculosis* Infection in free-roaming wild Asian elephant. *Emerg Infect Dis* 2017;23:555-557.

12. Davies GR, Neurmberger EL. Pharmacokinetics and pharmacodynamics in the development of anti-tuberculosis drugs. Tuberculosis 2008;88:S65-S74.

13. Davis M. *Mycobacterium tuberculosis* risk for elephant handlers and veterinarians. Parasitology international 2001;16:350-353.

14. Egelund EF, A Alsultan, CA Peloquin. Optimizing the Clinical Pharmacology of Tuberculosis Medications. Clinical Pharmacology & Therapeutics 2015; V98(4) Pp 387-393.

15. Egelund EF, Isaza R, Alsultan A, et al. Isoniazid and rifampin pharmacokinetics in two Asian elephants (*Elephas maximus*) infected with *Mycobacterium tuberculosis*. *J Zoo Wild Med* 2016;47:868-871

16. Feldman M, Isaza R, Prins C, et al. Point prevalence and incidence of Mycobacterium tuberculosis complex in captive elephants in the United States. Veterinary quarterly 2013;33:25-29.

17. Filippini P, Iona E, Piccaro G, et al. Activity of drug combinations against dormant *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2010;54:2712-2715.

18. Garcia-Tapia A et al. Action of Fluoroquinolones and Linezolid on Logarithmic-and Stationary-Phase Culture of Mycobacterium tuberculosis. Chemotherapy 2004; 50: 211-213.

19. Gumbo T, et al. Pharmacodynamic Evidence that Ciprofloxacin Failure Against Tuberculosis is not Due to Poor Microbial Kill but Rapid Emergence of Resistance. Antimicrobial Agents and Chemotherapy. 2005 V 49(8)Pp 3178-3181.

20. Greenwald R, Lyashchenko O, Esfandiari J, et al. Highly accurate antibody assays for early and rapid detection of tuberculosis in African and Asian elephants. Clinical and vaccine immunology 2009;16:605-612.

21. Griffin J, Buchan G. Aetiology, pathogenesis and diagnosis of *Mycobacterium bovis* in deer. Veterinary Microbiology 1994;40:193-205.

22. Heymann DL. Control of communicable diseases manual. Washington, D.C.: American Public Health Association; 2008.

23. Isaza R, Ketz C. A trunk wash technique for the diagnosis of tuberculosis in elephants. In: Verhandlungsbericht des Internationalen Symposiums uber die Erkrankungen der Zootiere, Vienna 1999;121-124.

24. Isaza R, Wiedner E, Hiser S, et al. Reference intervals for acute phase protein and serum protein electrophoresis values in captive Asian elephants (*Elephas maximus*). Journal of Veterinary Diagnostic Investigation 2014;26:616-621.

25. Lacasse C, Terio K, Kinsel MJ, et al. Two cases of atypical mycobacteriosis caused by Mycobacterium szulgai associated with mortality in captive African elephants (*Loxodonta africana*). Journal of zoo and wildlife medicine 2007;38:101-107.

26. Lambert M-L, Hasker E, Van Deun A, et al. Recurrence in tuberculosis: relapse or reinfection? The Lancet infectious diseases 2003;3:282-287.

27. Landolfi JA, Miller M, Maddox C, et al. Differences in immune cell function between tuberculosis positive and negative Asian elephants. Tuberculosis 2014;94:374-382.

28. Landolfi, JA, et al. Pulmonary tuberculosis in Asian Elehpants (Elephas maximus): Histologic Lesions with Correlation to Local Immune Response. Vet Path. 2015 Vol 52(3) pp535-542.

29. Larsen RS, Salman MD, Mikota SK, et al. Evaluation of a multiple-antigen enzyme-linked immunosorbent assay for detection of *Mycobacterium tuberculosis* infection in captive elephants. Journal of zoo and wildlife medicine 2000;31:291-302.

30. Léçu A, Ball R. Mycobacterial infections in zoo animals: relevance, diagnosis and management. International zoo yearbook 2011;45:183-202.

31. Leibert E, Rom WN. New drugs and regimens for treatment of TB. *Expert Rev Anti-Infect Ther* 2010;8:801-813.

32. Lewerin, SS, Olsson S-L, Eld K, Roken B, Ghebremichael S, Koivula T, Kallenius G, Bolske G. Outbreak of *Mycobacterium tuberculosis* infection among captive Asian elephants in a Swedish zoo. *Vet Rec* 2005;156:171-175.

33. Magnuson RJ, et al. Rapid Screening for Mycobacterium tuberculosis Complex in Clinical Elehpant Trunk Wash Samples. Research in Vet Science 2017; 112 Pp52-58.

34. Maslow JN, Mikota SK, Zhu M, et al. Population pharmacokinetics of isoniazid in the treatment of *Mycobacterium tuberculosis* among Asian and African elephants (*Elephas maximus* and *Loxodonta africana*). Journal of veterinary pharmacology and therapeutics 2005;28:21-27.

35. Maslow JN, Mikota SK, Zhu M, et al. Pharmacokinetics of ethambutol (EMB) in elephants. Journal of veterinary pharmacology and therapeutics 2005;28:321-323.

36. Michalak K, Austin C, Diesel S, et al. *Mycobacterium tuberculosis* infections as a zoonotic disease: transmission between humans and elephants. Emerging infectious diseases 1998;4:283-287.

37. Mikota SK, Maslow JN. Tuberculosis at the human-animal interface: An emerging disease of elephants. Tuberculosis 2011;91:208-211.

38. Mikota SK, Peddie L, Peddie J, et al. Epidemiology and diagnosis of *Mycobacterium tuberculosis* in captive Asian elephants (*Elephas maximus*). Journal of zoo and wildlife medicine 2001;32:1-16.

39. Montali RJ, Mikota SK, Cheng LI. *Mycobacterium tuberculosis* in zoo and wildlife species. Review of science and technology 2001;20:291-303.

40. Murphree R, Warkentin JV, Dunn JR, et al. Elephant-to-human transmission of tuberculosis, 2009. Emerging infectious diseases 2011;17:366-371.

41. National Association of State Public Health Veterinarians. Compendium of measures to prevent disease associated with animals in public settings, 2005. MMWR Recommendations and reports: Morbidity and mortality weekly report Recommendations and reports/Centers for Disease Control 2005;54:1.

42. National Tuberculosis Controllers Association. Guidelines for the investigation of contacts of persons with infectious tuberculosis. Recommendations from the National Tuberculosis Controllers Association and CDC. MMWR Recommendations and reports: Morbidity and mortality weekly report, Recommendations and reports/Centers for Disease Control 2005;54:1.

43. NIOSH. Health hazard evaluation report: evaluation of potential employee exposure to Mycobacterium tuberculosis at an elephant refuge. By Neimeier RT. MeadK, dePerio MA, Martin SB, Burr GA Cincinnati, OH: U.S. Department of Health and Human Services, Centers For Disease Control and Prevention, National Institute for Occupational Safety and Health; 2015.

44. Obanda V. First reported case of Fatal tuberculosis in a wild African elephant with past human-wildlife contact. Epidemiology and Infection 2013; 141(7): 1476-1480.

45. Oh P, Granich R, Scott J, et al. Human exposure following *Mycobacterium tuberculosis* infection of multiple animal species in a metropolitan zoo. Emerging infectious diseases 2002;8.

46. Papastavros T, Dolovich LR, Holbrook A, et al. Adverse events associated with pyrazinamide and levofloxacin in the treatment of latent multidrug-resistant tuberculosis. CMAJ 2002;167:131-136.

47. Payeur J, B., Jarnagin JL, Marquardt JG, et al. Mycobacterial isolations in captive elephants in the United States. Annals of the New York Academy of Sciences 2002;969:256-258.

48. Peloquin CA, Maslow JN, Mikota SK, et al. Dose selection and pharmacokinetics of rifampin in elephants for the treatment of tuberculosis. *J Vet Pharm Ther* 2006;29:581-585.

49. Perera BVP, Salgadu MA, Gunawardena GSPdS, et al. First confirmed case of fatal

tuberculosis in a wild Sri Lankan elephant. *Gajah* 2014;41:28-31.

50. Saukkonen JJ, Cohn DL, Jasmer RM, et al. An official ATS statement: hepatotoxicity of antituberculosis therapy. American journal of respiratory and critical care medicine 2006;174:935-952.

51. Scheftel JM, Elchos BL, Cherry B, et al. Compendium of veterinary standard precautions for zoonotic disease prevention in veterinary personnel: National Association of State Public Health Veterinarians Veterinary Infection Control Committee 2010. Journal of the American Veterinary Medical Association 2010;237:1403.

52. Simpson G, Zimmerman R, Shashkina E, et al. *Mycobacterium tuberculosis* infection among Asian Elephants in captivity. *Emerg Infect Dis* 2017;23:513.

53. Spigelman MK. New tuberculosis therapeutics: a growing pipeline. *J Infect Dis* 2007;196:S28-S34.

54. Stephens N, Vogelnest L, Lowbridge C, et al. Transmission of Mycobacterium tuberculosis from an Asian elephant (Elephas maximus) to a chimpanzee (Pan troglodytes) and humans in an Australian zoo. Epidemiology and infection 2013;141:1488-1497.

55. Thompson NP, Caplin ME, Hamilton MI, et al. Anti-tuberculosis medication and the liver: dangers and recommendations in management. European respiratory journal 1995;8:1384-1388.

56. Verma-Kumar S, Abraham D, Dendukuri N, et al. Serodiagnosis of Tuberculosis in Asian Elephants (*Elephas maximus*) in Southern India: A Latent Class Analysis. PLOs one 2012;7.

57. Vogelnest L. Tuberculosis: an emerging zoonosis. New South Wales Public Health Bulletin 2013;24:32-33.

58. Vogelnest L, Hust F, Vinette HK, et al. Diagnosis and management of tuberculosis (*Mycobacterium tuberculosis*) in an Asian elephant in Australia. In: Mycobacterial diseases of wildlife 2012;242.

59. Wiedner E, RP Hunter. Antimicrobial Drug Use in Zoological Animals. In Antimicrobial therapy in Veterinary Medicine 5th ed. editors S Giguere, JF Prescott and PM Dowling. 2013 John Wiley & Sons. Inc.

60. Wiedner E, Schmitt DL. Preliminary report of side effects associated with drugs used in the treatment of tuberculosis in elephants. In: Proceedings of the international elephant foundation meeting, Orlando, FL 2007;15-20.

61. Wilson E, Mikota S, Bradford JP, et al. Seropositive, culture negative tuberculosis in an Asian elephant (*Elephas maximus*). In: AAZV, AAWV Joint Conference, South Padre Island, Texas 2010;170.

62. 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.

63. 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.

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

65. Zlot A, Vines J, Nystrom L, et al. Diagnosis of Tuberculosis in Three Zoo Elephants and a Human Contact—Oregon, 2013. *MMWR* 2016;64:1398-1402.

Appendix 2: Trunk Wash Technique (Isaza and Ketz): 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^{o} C) until it can be sent to the laboratory. Often the recommended three daily culture 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. Therefore, 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 though 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. 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, 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 3: CVI Form Example

AMERICAN ASSOCIATION of ZOO VETERINARIANS

STANDARD CERTIFICATE OF VETERINARY INSPECTION

Nº 99292

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

CONSIGNEE Joe's Elephant Land
ADDRESS 345 Elephant Utopia Lane
Elephantville, LA.
PHONE (314) 123-3456

DATE Issued 01/01/2016
Mode of Transport ☒ Land ☐ Air ☐ Sea
NAME OF AGENT

ANIMAL I.D. Tatoo Band Tag Etc.	NAME Common Scientific	PHYSICAL DESCRIPTION Age Sex Weight Other
Jumbo	Asian Elephant Elephas maximus	45 years old Female 8500 lbs.
Mabel	Asian Elephant Elephas maximus	42 years old Female 7700 lbs.

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 Site of injection
☐ Mammalian old tuberculin
☐ Bovine PPD results: ☐ Pos ☐ Neg ☐ Suspicious
Other (specify)
EIA results for Equine ☐ Positive ☐ Negative
Brucellosis Test for Ruminants

Test Dilution			Results
1-50	1-100	1-200	

Mtb TST. Trunkwash culture see details below

Method of Examination ☐ Visual ☑ Physical

Jumbo - Mtb Category A -
Trunkwash history
Triple TW Jan 3-5, 2015, Lab: NVSL, Results: Neg
Triple TW May 13-17, 2014, Lab: NVSL, Results: Neg
Mabel - Mtb Category A
Triple TW. Jan 3-5, 2015, Lab: NVSL, Results: Neg
Triple TW, May 13-17, 2014, Lab: NVSL, Results: Neg

Permit Obtained If Required Yes ☒ No ☐

"I certify, as an accredited 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 tests listed were as indicated on the certificates. To the best of my knowledge, the animals listed on this certificate meet the state of destination and federal interstate requirements. No further warranty, express or implied."

Signature Joe Zoodel DVM
Printed Name Joe Zoo Vet
Approved by John State Vet
State Veterinarian

National Vet. Accrediation Prog. I.D.: 1234
State Veterinary License No. 1235 AR
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.

White Copy – State Veterinarian Office
Green Copy – State Veterinarian Office
Yellow Copy – Issuing Veterinarian
Pink Copy – Accompany Shipment
Orange Copy – Owner

Camden Printing 16063 - Rev. 07/12

Appendix 4: Laboratories for Mtb culture samples:

Suggested Certified Laboratories for Mycobacteria Cultures and Research status qPCR on Trunk Washes.

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.

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: Lab Contact for Therapeutic Drug Level Monitoring

INFECTIOUS DISEASE PHARMACOKINETICS LABORATORY

Contact: Dr. Charles Peloquin

1600 SW Archer Rd., P4-30
Gainesville, FL 32610
Phone: 352-273-6710 Fax: 352-273-6804
E-mail: peloquinlab@cop.ufl.edu
Website: http://idpl.pharmacy.ufl.edu

Direct link to IDPL with Submission Form

https://cop-idpl.sites.medinfo.ufl.edu/files/2017/06/IDPL-UFHealth-v05.17.pdf

Appendix 6: Epidemiological 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). Sensitivity = [TP ÷ (TP + FN)] X 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. Specificity = [TN ÷ (TN + FP)] X 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.

Pharmacokinetics (PK): How a drug behaves in the body; i.e. how much needs to be given to obtain a particular drug level, where and how the drug is metabolized, how long the drug lasts in the system.

Pharmacodynamics (PD): The action of the drug; i.e., whether it actually kills infectious organisms, whether it causes adverse effects.

Tmax: refers to the time after administration of a drug when the maximum plasma concentration is reached. This needs to be determined when serum drug levels are measured.

Appendix 7: 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.
- NIOSH [2015]. Health hazard evaluation report: evaluation of potential employee exposures to *Mycobacterium tuberculosis* at an elephant refuge. By Niemeier RT, Mead K, de Perio MA, Martin SB, Burr GA. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HHE Report No. 2010-0080-3235. Available at https://www.cdc.gov/niosh/hhe/reports/pdfs/2010-0080-3235.pdf.
- 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/bmbl5/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
- Protect Yourself Against Tuberculosis – A Respiratory Protection Guide for Health Care Workers (http://www.cdc.gov/niosh/docs/96-102/pdfs/96-102.pdf) – resource booklet for employees and outlines in Appendix II what is required in a respiratory protection program
- OSHA's Respiratory Protection informational booklet (https://www.osha.gov/Publications/OSHA3079/osha3079.html or https://www.osha.gov/Publications/osha3079.pdf) – about OSHA Respiratory Protection Programs
- OSHA's Small Entity Compliance Guide for the Revised Respiratory Protection Standard (https://osha.gov/Publications/3384small-entity-for-respiratory-protection-standard-rev.pdf) – Explains all parts of an appropriate respiratory protection program and how to comply. Also contains a sample respiratory protection program in Attachment 4 that can be used as a model program.
- The Washington State Department of Labor and Industries has a user-friendly, fillable template that is helpful in developing a respiratory protection program at http://www.lni.wa.gov/Safety/TrainingPrevention/Programs/Respiratory.asp.
- *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

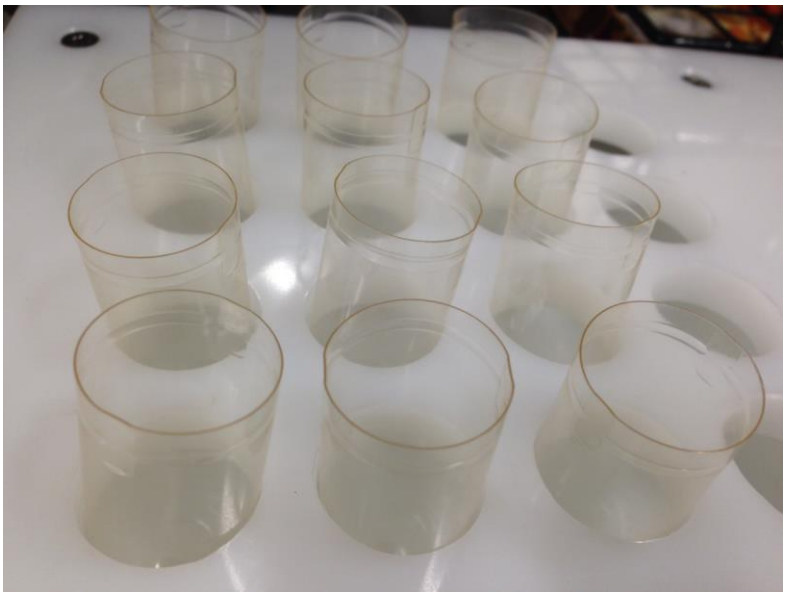
Appendix 8;
Novel Method for Making Oral Capsules for Rifampin and Ethambutol Administration.
Note: It is important that all elephants receive routine training for basic veterinary procedures such as taking oral medications before they have a need to take oral medications if at all possible. Though this method worked well in an elephant, it is not a guarantee that such a recipe will be accepted by all elephants. Blood drug levels for RIF and ETH were verified after dosing with these capsules and found to be adequate at the institution where this elephant was treated. The elephant was trained initially to accept oral capsules by using un-medicated coconut oil filled Size 10 gelatin capsules first. Such training is a crucial portion of successful treatment.

Coconut Oil Capsules:
Materials:

- **Size 10 (18ml) capsules ***
- **Chocolate Melter** (this is table top warmer)
- **Coconut Oil** (melt on 'warm' setting)
- **Rifampin powder measured out into individual doses**
- **Ethambutol powder or tablets measured out into individual doses**
- **Tongue Depressors or silicone spatula**
- **60cc catheter tip syringe**
- **Gloves**
- **Capsule Tray** (this is homemade piece of high density polyethylene with capsule sized holes drilled in it. (Seen in figures 4-5)

Directions: (see Illustrations below):

Figure 1. Open empty capsules. Place 12 'bottoms' (larger half of each capsule) into holes of homemade capsule tray or test tube rack. Set 'tops' aside for now. The number of capsules needed will be determined by the ethambutol dose.



*TORPAC size 10 (18ml) capsules, http://www.torpac.com/reference/sizechart/

10. Wipe off all rifampin mix residue from each capsule using clean cloth (Fig. 9)

11. Place all capsules that together make one dose in a brown Ziploc bag and add 10ml of liquid coconut oil to the bag. Seal bag and kneed it lightly to coat the capsules with the oil. Label each dose and place into freezer. (Fig 10-11).

*TORPAC size 10 (18ml) capsules, Empty gelatin capsules that can be filled
http://www.torpac.com/reference/sizechart/

Figure 1: ½ of gelatin capsule set in homemade capsule tray

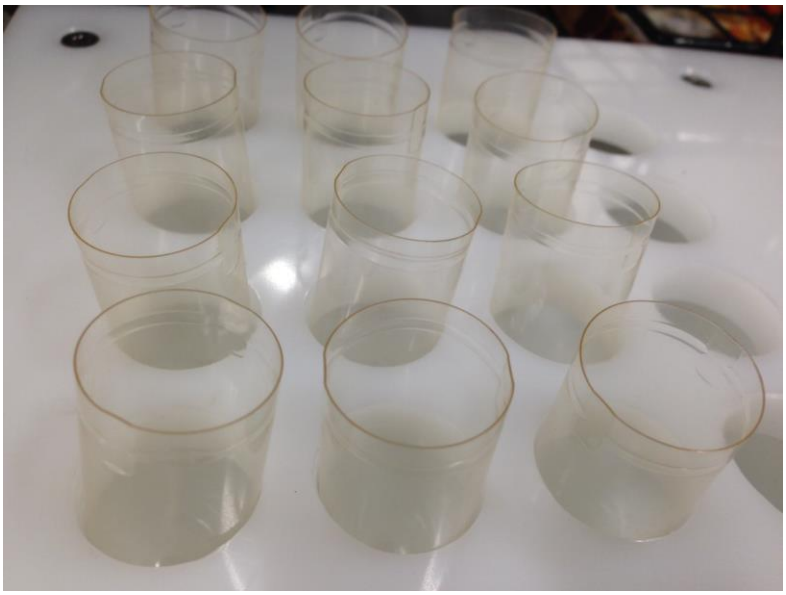


Figure 2-3: adding warm coconut oil to RIF dose in mixing container

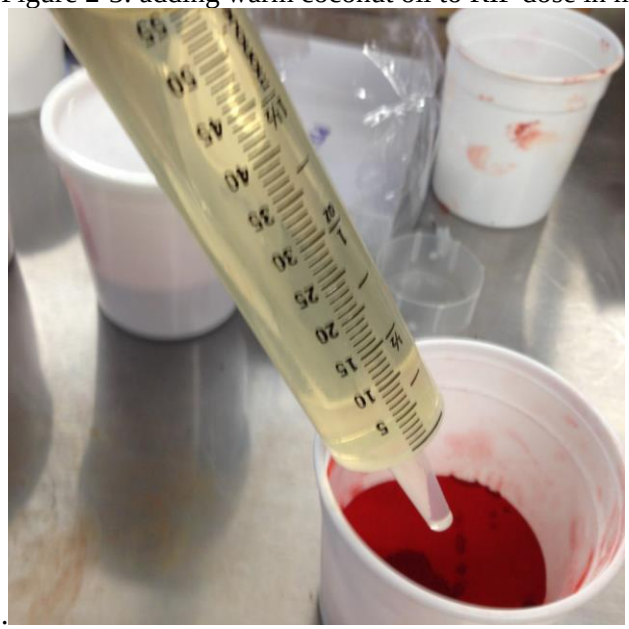


Figure 4: Pour coconut oil/RIF mix into bottom half of gel capsules

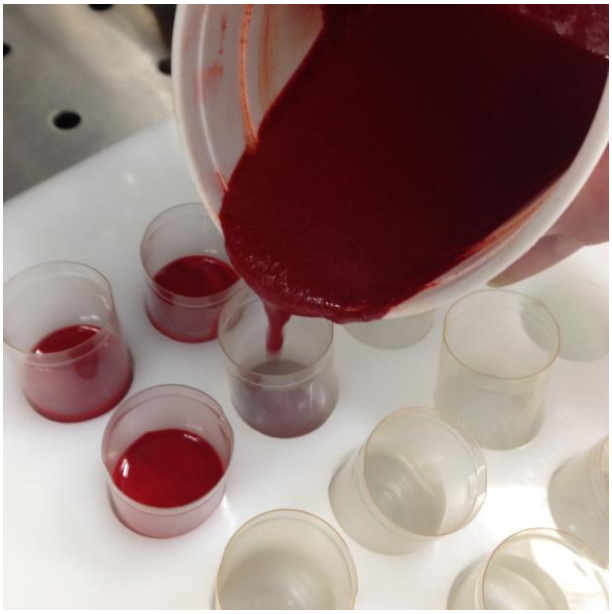


Figure 5:Add ETH tablets to gel capsule 1/2 .

Figure 6: Get all of RIF dose from container by adding addition 20 mls of clean coconut oil and swirl or stir.

Figure 7: Carefully top off capsules with last of RIF dose. Then refrigerate capsules in rack for 10 minutes to help solidify coconut oil

Figure 8: Carefully place top half of gel capsule onto filled bottom.

Figure 9. Wipe all residue off of capsule with soft towel.

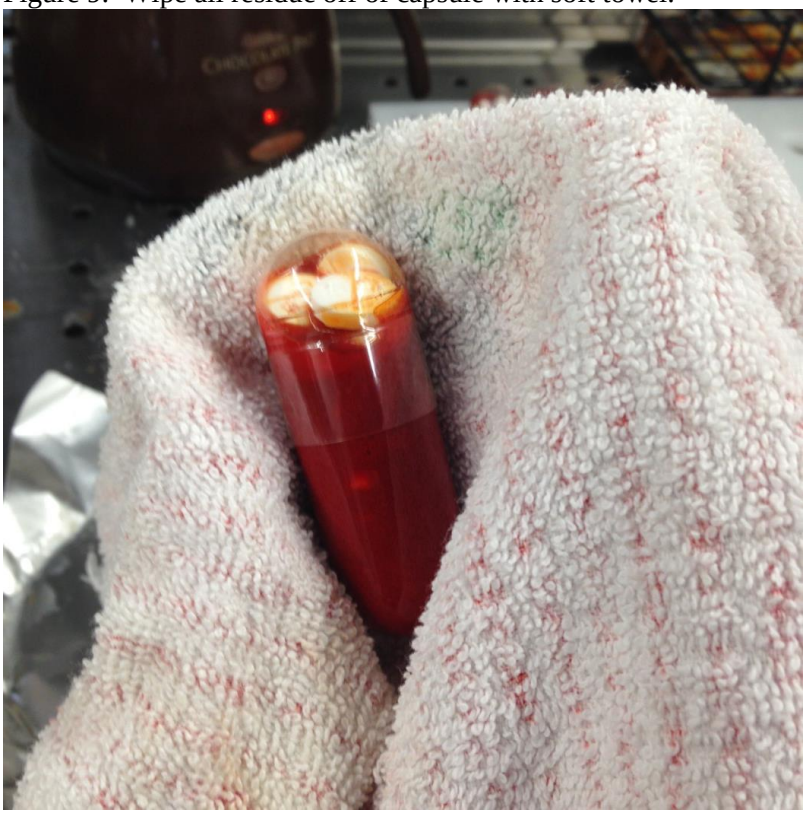


Figure 10-11: Place all capsules that together make one dose in a brown Ziploc bag and add 10ml of liquid coconut oil to the bag. Seal bag and kneed it lightly to coat the capsules with the oil. Label each dose and place into freezer.

Appendix 9. Participants of 2016 Workshop for Input into 2017 Recommendations

5th Annual Management and Research Priorities of Tuberculosis for Elephants in Human Care Workshop
24 July 2016.

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*Attended Via Webinar