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Review of Tuberculosis in Captive Elephants and Implications for Wild Populations

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Historical perspective

Tuberculosis (TB) is an ancient disease of animals and humans. Clinical signs, characteristic of TB were described in Asian elephants by Ayurvedic physicians over 2000 years ago (Iyer 1937; McGaughey 1961). TB has been reported in captive elephants worldwide (Narayanan 1925; Bopayya 1928; Seneviratna *et al.* 1966; Pinto *et al.* 1973; Chandrasekharan *et al.* 1995; Mikota *et al.* 2000, 2001; Ratanakorn 2001; Gavier-Wieden *et al.* 2002; Chakraborty 2003; Rahman 2003; and others).

Elephant TB surveillance began in the U.S. in 1997. Between 1996 and 2007 at least 40 elephants representing over 12% of the Asian elephants in the United States have been diagnosed with culture positive TB, primarily *Mycobacterium tuberculosis,* the human form. This figure is significant considering the low prevalence of human TB in the U.S. (< 4 persons / 100,000) compared to Asia (Table 1). Seven elephant TB cases have been reported in Europe (Lewerin *et al.* 2005; Moller *et al.* 2005, 2006).

Intermingling of captive elephants with wild elephants, domestic livestock, and humans is common in many Asian range countries. The potential for TB transmission exists when wild bulls breed captive cows, when grazing land is shared with livestock, or when captive elephants are exposed to infected humans (Mikota *et al.* 2006a, 2006b).

Table 1. Estimated prevalence of human TB in the USA and selected Asian elephant range countries for the year 2006 (WHO 2008).

Country	Prevalence/100,000
USA	5
India	299
Indonesia	253
Myanmar	169
Nepal	244
Sri Lanka	80
Thailand	197

TB is a global epidemic for humans and an estimated 1.7 million people died of TB in 2006 (WHO 2008). The World Health Organization (WHO) states that early diagnosis and treatment is the best way to control TB, thus reducing the impact on infected individuals and their ability to transmit the disease.

TB lessons from Africa and mastodons

African buffalo have been under surveillance for TB (caused by *M. bovis*) since 1990. In a 15-year period, TB spread to infect all but the most northern herds, spilled over to 10 other mammalian species and is now found in several adjacent game reserves. Mortalities due to advanced TB occur at an annual rate of 11% (Michel *et al.* 2006). A decrease in overall body condition has been correlated with increased TB prevalence (Caron *et al.* 2003). Due to the chronic nature of TB, the long-term impact on this species has yet to be determined.

M. tuberculosis has recently been introduced into free-ranging banded mongooses (*Mungos mungo*) in Botswana and suricates (*Suricata suricatta*) in South Africa, suggesting that free-ranging African elephants, as well as Asian, may also be at risk for infection with human TB (Alexander *et al.* 2002).

Pathognomonic tuberculous lesions were identified in 59 of 113 mastodon (*Mammut americanum)* skeletons (52%) implicating pandemic TB as one of several probable factors contributing to mastodon extinction (Rothschild *et al.* 2006).

Causative agent

TB is caused by a bacterium in the genus *Mycobacterium*. Mycobacteria infect a broad range of species (Montali *et al.* 2001). *Mycobacterium tuberculosis* (*M. tb*) is the predominant disease-causing agent in elephants in North America and Europe, although cases due to the bovine form (*M. bovis*) have occurred. *Mycobacterium avium* (bird form) and saprophytic non-tuberculous mycobacteria (NTM) are commonly isolated from elephant trunk wash samples (Payeur *et al.* 2002). Only *M. szulgai*, an unusual NTM has been associated with disease in elephants (Lacasse *et al.* 2007).

Both African and Asian elephants are susceptible to TB although the disease appears to be more common in Asian elephants. This may reflect the closer association between Asian elephants and humans rather than a species predisposition.

Transmission and pathogenesis

Transmission of TB occurs primarily via the respiratory route. Feces, urine, genital discharges, milk, and feed or water may contain contaminated droplets. In elephants, *M. tb* has been isolated from respiratory secretions, trunk washes, feces, and vaginal discharges.

TB exposure may result in various outcomes, listed in Table 2. Latent TB infection (LTB) is the absence of clinical disease and no evidence of active shedding of live organisms. In LTB, the bacteria are sequestered in lung granulomas that may reactivate at a later date. Individuals with LTB are a reservoir for future active cases. Although only 5-10% of latently infected humans with normal immune status will develop active TB during their lifetime (CDC 2007) the identification and treatment of those at high activation risk remains an effective means of control (Nuermberger *et al.* 2004). It is unknown whether LTB occurs in elephants.

Clinical signs

TB in elephants cannot be diagnosed only on the basis of clinical signs which may be absent until the disease is quite advanced. Seemingly healthy elephants may harbor and transmit TB to other elephants and humans. In 24 culture-confirmed TB cases (17 in the US and 7 in Europe) only three elephants showed signs before diagnosis (Konstantin Lyashchenko, pers. comm.). Clinical signs, when present, may include weight loss, weakness, coughing or difficulty breathing. Many other diseases may cause similar signs. Respiratory discharges from the trunk are occasionally noted. Exercise intolerance may be seen in working elephants. Elephants that show antemortem wasting may have disseminated disease on necropsy.

Guidelines for the control of TB in elephants

The guidelines for the control of tuberculosis in elephants, which specify criteria for diagnosis, surveillance, and treatment were instituted in the U.S. in 1997. The guidelines are revised as new information becomes available. The 2008 Guidelines will become official pending approval (in October 2008) by the board of directors of the United States Animal Health Association (USAHA) the current overseeing body. The 2008 draft guidelines are posted on the USAHA web site (www.usaha.org/committees/tb/DraftTBGuidelines.pdf).

Diagnosis

The diagnosis of TB in elephants has been problematic. Radiography is not feasible and

Table 2. Possible scenarios following exposure to TB.

Scenario	Outcome
1	All bacteria are killed and no disease results
2	Bacteria multiply and clinical disease results (primary TB)
3	Infection and eradication
4	Bacteria become dormant and never cause disease (LTB)
5	Dormant organisms reactivate and cause active disease (often associated with immune suppression or concurrent disease)

many techniques commonly used in humans (sputum examination and culture, intradermal tuberculin testing) are unreliable in elephants. The ElephantTB STAT-PAK® Assay (Chembio Diagnostic Systems Inc., Medford, NY; www.chembio.com), a serological test licensed in August 2007 in the U.S. opens the door to testing in range countries.

ElephantTB STAT-PAK® Assay and Multi-Antigen Print Immunoassay (MAPIA)™

The STAT-PAK® Assay is a qualitative, immunochromatographic screening test for the detection of antibodies to *M. tuberculosis* and *M. bovis* in serum, plasma, or whole blood (Lyashchenko *et al.* 2006). The Assay uses a unique cocktail of recombinant *M. tuberculosis* proteins bound to a membrane solid phase. Blue latex particles conjugated with protein are used as the detection system. A drop of test sample and three drops of diluent are applied to the test well and flow through the membrane strip. If antibodies are present, the test line will appear blue (Fig. 1).

The MAPIA™ is a confirmatory test (Lyashchenko *et al.* 2000, 2006). Elephant serum samples are incubated with a MAPIA strip and antigen-bound antibodies are visualized using a specific IgG-binding enzyme conjugate and corresponding substrate. The MAPIA is a laboratory test that is currently available only in the U.S through Chembio Diagnostic Systems.

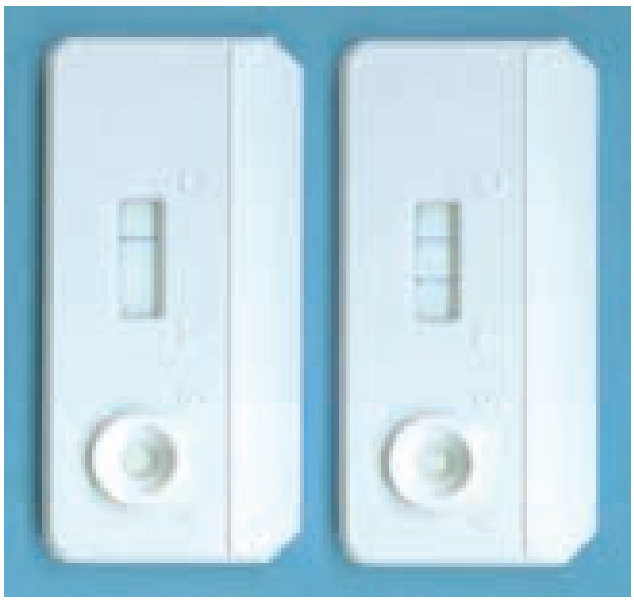

Figure 1. ElephantTB STAT-PAK® Assay. Left: non-reactive test; right: reactive test.

To date 99 Asian and 72 African elephants in Europe, Australia, South Africa, and the U.S. have been tested using the ElephantTB STAT-PAK® Assay and MAPIA including 23 elephants with culture-confirmed TB. Preliminary data has demonstrated 100% sensitivity and 97% specificity for STAT-PAK® Assay and 100% sensitivity and 100% specificity for the MAPIA using culture as the reference standard.

If the STAT-PAK® Assay is used as a screening test and the MAPIA™ is sequentially applied as a confirmatory assay, the accuracy of this testing algorithm is 100%. Seroconversion on the STAT-PAK® Assay and MAPIA™ has been noted in several elephants months to years prior to a positive culture (Lyashchenko *et al.* 2006).

An important advantage of serodiagnosis compared to trunk wash culture is that, once established, the antibody response remains sustained whereas culture may be intermittently positive or negative in infected elephants. A decline in specific antibodies to certain antigens in MAPIA has been observed suggesting a possible method to monitor response to therapy (Lyashchenko *et al.* 2006).

Although the STAT-PAK® Assay is new, retrospective studies (looking at banked serum from confirmed cases) have shown the test to be an accurate and early TB predictor, often preceding culture detection by months to years. This presents an opportunity to segregate infected elephants and initiate treatment before shedding and transmission to other elephants or people.

Commercial distribution of Chembio's ElephantTB STAT-PAK® Assay in the U.S. is currently restricted to the National Veterinary Services Laboratories (NVSL) and the test performed by NVSL personnel. The STAT-PAK was developed as a point-of care test, however, and is available internationally. Contact Chembio Diagnostic Systems, Inc. for further information (www.chembio.com).

Culture

Samples for culture are obtained using a trunk wash technique. Sterile saline is instilled and the trunk is elevated then lowered into a zippered plastic bag or other clean collection device. Ideally the elephant is trained to forcibly exhale so that the sample is from the lower respiratory tract. The sample is transferred to a secure screw-top tube and submitted to a certified TB laboratory. Three samples are collected on separate days. The Guidelines include a description of the procedure. A modified procedure using a tray has been developed in Asia for trunk-phobic elephants (Abraham & Davis 2008).

Although isolation of the organism is the "gold standard" to diagnose TB, culture has inherent limitations as a primary diagnostic technique: 1) Failure to isolate the organism does not rule out infection as characteristic intermittent shedding provides a potential for false-negative results. 2) Sample quality from the trunk wash method is variable and contamination is common. 3) Reporting time is slow, typically eight weeks. Infected elephants that are shedding while culture results are pending pose a risk to other elephants and humans. 4) Culture (requiring three samples per elephant) may not be practical or affordable to screen large numbers of elephants in Asia.

In Sweden, only six of 174 trunk wash samples were culture positive pre-mortem from five elephants confirmed TB positive (by culture) postmortem. All five elephants tested positive by the STAT-PAK® Assay (Moller *et al.* 2006).

Adequate laboratory support for culture may not be available in range countries. Techniques that have evolved in U.S. labs to address the issue of contamination may not be readily adopted in labs that have human TB diagnosis and control as their primary mission. These modified techniques developed by the National Veterinary Services Laboratories (Ames, Iowa, USA) are included in the guidelines.

Despite limitations, culture is an important diagnostic technique. Speciation (to differentiate *M. tuberculosis* and *M. bovis*) may be pertinent to management strategies. Drug sensitivity testing is important if treatment is planned.

Other tests

Intradermal tuberculin test: The intradermal tuberculin test correlates poorly with culture results and is not reliable in elephants (Mikota *et al.* 2001; Lewerin *et al.* 2005).

Acid-fast stain (AFS): A positive AFS is suggestive of TB but not definitive. AFS has low sensitivity (50% in humans) and is non-specific, particularly in geographic areas where NTM are commonly isolated (Dalovisio *et al.* 1996). AFS has not been widely used to detect TB in elephants.

Nucleic acid amplification techniques: The Gen-Probe Amplified Mycobacterium tuberculosis Direct Test (MTD; Gen-Probe, San Diego, California, 92121) detects RNA from live or dead TB organisms. In the U.S. the MTD is approved for the diagnosis of TB in humans but only in conjunction with culture. The MTD is quick (2.5 to 3.5 hours) and can detect low numbers of organisms. The MTD has been used in a limited number of elephant studies (Payeur *et al.* 2002).

Enzyme Linked Immunosorbent assay (ELISA): The ELISA measures antibodies against specific antigens. One ELISA study demonstrated an estimated sensitivity of 100% and specificity of 100%, on 47 elephants (7 culture positive) (Larsen *et al.* 2000). The ELISA is not available commercially.

Restriction fragment length polymorphism (RFLP): Commonly called DNA finger-printing, RFLP can identify different mycobacterial strains. Six *M. tb* strains were identified from six U.S. herds (Mikota *et al.* 2001) and five elephants and one giraffe were infected by four different *M. tb* strains in Europe (Lewerin *et al.* 2005).

Postmortem examination

Deceased elephants should undergo a complete postmortem examination. A number of guidelines are available for this purpose:

1) Techniques and Procedures for Post-mortem of Elephants (2003) Cheeran, J.V. and Nair, N.D. Project Elephant, Ministry of Environment & Forests, Government of India, New Delhi.

2) Elephant Necropsy Protocol of the American Zoo and Aquarium Association Elephant Species Survival Plan (www.elephantcare.org/protodoc_files/new%2006/ElephNecropsy2004_2005_final_version.pdf)

3) A guide to post-mortem procedure and a review of pathological processes identified in the elephant by D.F. Keet & R.G. Bengis (www.elephantcare.org/protodoc_files/afrnecro.pdf)

Pathologic changes in TB infected elephants are found primarily in the lungs and thoracic lymph nodes, although extrapulmonary or disseminated TB may involve the liver, kidney, spleen, adrenals, or genitourinary tract. Pulmonary lesions may be focal or widespread, depending on the disease stage. In advanced cases, extensive caseocalcareous and cavitating lesions may be seen throughout the lungs often associated with large pulmonary abscesses colonized by secondary bacteria (Mikota *et al.* 2000). Enlargement of bronchial and thoracic lymph nodes is common.

Respiratory protective equipment should be available during all elephant necropsy procedures. To protect against TB, properly fitted, disposable, particulate filter respirators that are rated to protect against TB or positive air pressure respirators (PAPRs) should be used. Ordinary surgical masks do not protect against TB. PAPRs are expensive cumbersome to wear and may not be practical during the monsoon in Asia.

Approaching the thorax through the diaphragm will minimize exposure to TB (Montali 2006).

Treatment

Elephant treatment guidelines in the U.S. have evolved from protocols known to cure TB in humans. Pharmacokinetic studies have been conducted in elephants for isoniazid (Maslow *et al.* 2005), ethambutol (Maslow *et al.* 2005), pyrazinamide (Zhu 2005), and rifampin (Maslow *et al.* 2005). The duration of treatment is 12 months. Three drugs are administered for two months followed by two drugs for 10 months using a combination of isoniazid (INH), pyrazinamide (PZA), rifampin (RIF), and ethambutol (ETH). Streptomycin has not been used in the U.S. Pyrazinamide is not effective against *M. bovis.*

Veterinarians seeking to treat elephants for TB should consult current Guidelines (www.usaha.org/committees/tb/DraftTBGuidelines.pdf) and experienced colleagues.

In the case of multi-drug resistant TB (MDR-TB), defined as resistance to both INH and RIF, second-line drugs such as amikacin, ciprofloxacin, levofloxacin and others may be needed. The increased risks to staff must be considered before initiating treatment for MDR-TB.

TB drugs may be given by direct oral or rectal administration. Adequate drug levels cannot be achieved if drugs are mixed with food offered free-choice. Some drugs are bitter and elephants will refuse them. Elephants can be trained to accept a bite block and medications delivered via a large animal dose syringe. Most elephants can also be readily trained to accept rectal administration; adequate blood levels can be achieved for INH and PZA (but not RIF) by this route. Treatment is challenging and elephants, like humans, may experience side effects (Dumonceaux & Mikota 2006). Therapeutic drug monitoring (Peloquin 2002) is recommended for elephants receiving anti-TB drugs but may not be available in all countries.

TB drugs are expensive and the cost to treat one elephant for a year in the U.S. may exceed US$ 50,000. The availability of cost effective drugs in Asia will make it possible to treat an elephant for under US$ 2000.

Zoonotic considerations and personnel health and safety

Several reports discuss the zoonotic aspects of elephant TB (Maslow 1997; Ryan 1997; Michalak

et al. 1998; Davis 2001; Montali *et al.* 2001; Oh *et al.* 2002). Because *M. tb* is primarily a human pathogen, exposure to infected humans is the most likely source of infection for elephants and other humans.

Infected elephants pose the greatest risk to mahouts who live in close daily association. The extent of risk to humans (or elephants) in other situations such as religious events or large festivals has not been studied. According to the U.S. Center for Disease Control (CDC 2008): "To become infected, a person usually has to spend a relatively long time in a closed environment where the air was contaminated by a person with untreated tuberculosis who was coughing and who had numerous *M. tuberculosis* organisms (or tubercle bacilli) in secretions…" Note however, that the World Health Organization (WHO 2007) says: "…When infectious people cough, sneeze, talk or spit, they propel TB germs, known as bacilli, into the air. A person needs only to inhale a small number of these to be infected…."

After the diagnosis of TB in one U.S. herd, 11 of 22 handlers had positive intradermal test results and one handler had active TB with the same RFLP pattern as the infected elephant (Michalak *et al.* 1998). This is the only published case of active human TB associated with elephant contact to date.

Annual TB testing is advisable for personnel working with elephants. Consultation with local health authorities is advisable and facilities should develop protocols to protect staff.

Management of TB in elephants in range countries

The varying elephant keeping systems and differing cultural attitudes pose challenges for elephant TB control in Asia. Consideration must be given to national and international laws, local resources, and tourism. Managing TB in government versus privately owned elephants may require different strategies.

Permanent segregation, treatment, and euthanasia are the three basic methods to control TB. Permanent segregation may be the best options for old elephants in which infections may be long-standing and cure unlikely. Treatment is difficult and better methods to confirm cure are needed. The MAPIA™ changes in response to treatment but this test is only available in the U.S. A negative culture series is supportive but limited by the factors mentioned above. Euthanasia may not be acceptable or legal in some areas or it may be mandated in others as was done by the government in Sweden.

Preliminary algorithms for managing various scenarios and for addressing human health concerns are presented in Figures 2-5. Figure 2 is a simplistic flow chart of possible options for culture positive elephants. Segregation is a logical first step. A permanent quarantine facility with protective barriers to prevent the intermingling of wild elephants may be possible in some areas. Detailed protocols for the protection of human health are essential. Euthanasia is undesirable but perhaps should be considered in cases of MDR-TB given the difficulty of treatment and increased risk to humans.

Figure 3 depicts the more challenging management of culture-negative / serologically positive elephants. Prophylactic treatment is an option - again depending on the number of cases and available funds. Age and exposure history are considerations. Monitoring for active disease is also another option. Protocols will need to be established to determine the frequency of culture and continued research on other ways to detect shedding is encouraged. The current lack of a fast and accurate method to determine shedding is a major drawback.

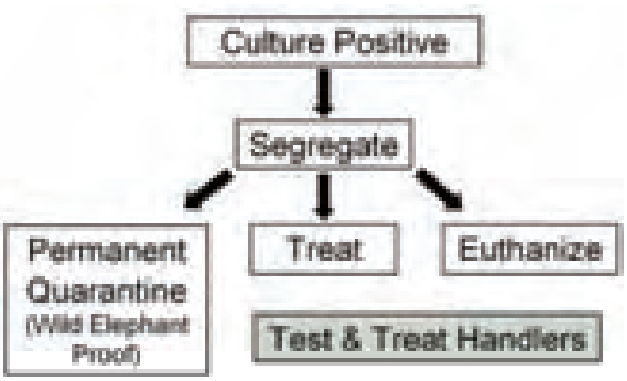

Figure 2. Options for culture positive elephants.

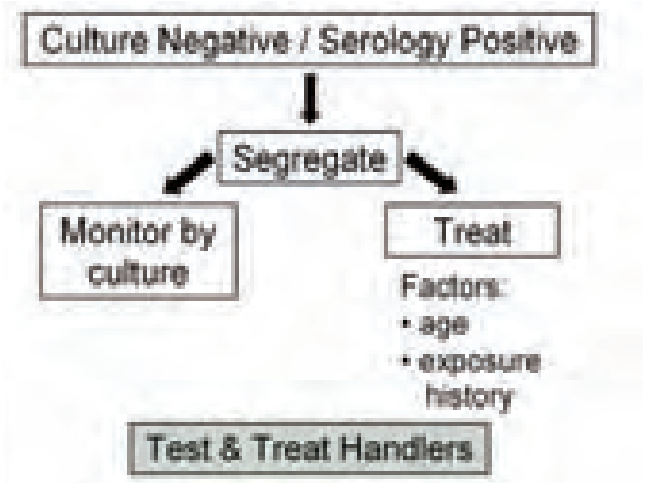

Figure 3. Options for culture negative / serology positive elephants.

Elephants that are serologically and culture negative but have had known exposure should be placed on an enhanced surveillance schedule because we do not yet know the time interval from infection to serological conversion (Fig. 4).

Figure 5 describes a protocol to address the TB health status of mahouts and other staff. This is a critical component of the overall strategy. Collaboration with public health agencies that already have TB screening programs will be helpful. All mahouts should be tested to initiate the program. Elephants cared for by infected mahouts should undergo increased monitoring.

Treatment considerations

It is unknown whether elephants develop latent TB. Of the 2 billion humans estimated to be infected with TB worldwide, 4-10% will develop active

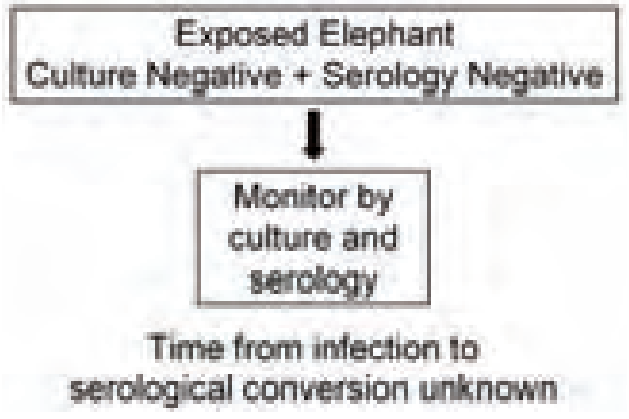

Figure 4. Options for exposed elephants that are culture and serology negative.

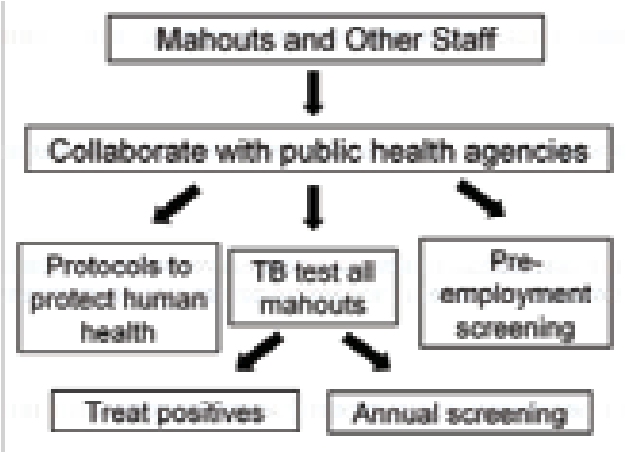

Figure 5. Care and treatment of staff.

disease (defined as TB infection with actively reproducing organisms); the other 90-96% will remain latent (the TB organisms are walled-off and sequestered in the body). However, latent TB can become active at any time, particularly if there is stress from other diseases or old age.

There are two approaches to consider to control TB in elephants:

1) Approach the problem aggressively and treat all serologically positive elephants even if they are culture negative.

Advantages:

- infected elephants may be treated before there is shedding and transmissibility to other elephants
- aggressively controlling TB among captive elephants may avert the introduction of TB into wild populations

Disadvantages:

- if elephants do develop latent disease, some elephants may be treated unnecessarily
- the lack of culture data precludes basing treatment on drug sensitivity testing and multi-drug resistant strains may be overlooked. There are, however, only a limited number of drugs to choose from, careful monitoring of treatment could help prevent creating resistance, and treatment of MDR TB (if present) may not be feasible anyway (cost prohibitive)
- the drugs are strong and some elephants will get sick during treatment (though no elephants have died during treatment)
- a positive culture is necessary for epidemiology studies (*M. tb* vs. *M. bovis*)

2) Wait until elephants are culture positive before initiating treatment.
Advantages:

- only elephants confirmed to be infected will be treated
- drug sensitivity information will be available

Disadvantages:

- infected elephants may transmit TB or succumb to infection prior to detection by culture
- the disease may become further advanced before detection, perhaps compromising the chances of successful treatment
- adequate laboratory support for culture may not be available
- infected elephants that go undiagnosed may develop exercise intolerance or other signs and be unable to work
- wild populations may be at increased risk if controlling TB among captive elephants is delayed

The majority of elephants that have died with TB in the U.S. have had advanced disease and were a source of infection for other elephants and humans. Despite 10 years of surveillance by culture, new cases are still being found. Perhaps if the STAT-PAK® Assay had been available earlier and had formed the basis of prompt treatment, we would have broken the cycle of infectious TB before now.

Deciding to treat serologically positive elephants might be a bold but necessary move if we are to control TB in elephants. In the early 1980's TB was thought to be under control in the U.S. and surveillance programs weakened. This led to a resurgence of TB between 1985 and 1992. Today we face forms of TB that cannot be treated because of inadequate global control programs.

TB and wild elephants

What are the risks that TB will infect wild populations or that it may already be there? Could TB become as devastating a problem for elephants as it is for humans? The risks are greater in regions where captive and wild elephants intermingle during grazing or breeding - India, Nepal and Myanmar for example. Communal grazing areas shared by elephants and domestic cattle pose an additional risk. Further studies of both captive and wild elephants are needed.

Management strategies are currently under development in Nepal that may serve as a model for other countries. A range country meeting (sponsored by WWF-Nepal and Elephant Care International) is tentatively planned for 2009 to share information and develop regional strategies.

References

For a comprehensive list of references on elephant TB see: www.elephantcare.org/TBrefs.htm

Abraham, D. & Davis, J. (2008) Revised trunk wash collection procedure for captive elephants in a range country setting. *Gajah* **28:** 53-54.

Alexander, K.A., Pleydell, E., Williams, M.C., Lane, E.P., Nyange, J.F.C. & Michel, A.L. (2002) *Mycobacterium tuberculosis*: An emerging disease of free-ranging wildlife. *Emerging Infectious Diseases* **8:** 598-601.

Bopayya, A.B. (1928) Tuberculosis in an elephant. *Indian Veterinary Journal* **5:** 142-145.

Caron, A., Cross, P.C. & du Toit, J.T. (2003) Ecological implications of bovine tuberculosis in African buffalo herds. *Ecological Applications* **13:** 1338–1345.

CDC (2007) *Fact Sheet: The Difference Between Latent TB Infection and Active TB Disease.* Centers for Disease Control and Prevention. www.cdc.gov/TB/pubs/tbfactsheets/LTBIandActiveTB.htm.

CDC (2008) *CDC Health Information for International Travel 2008. Chapter 4 – Prevention of Specific Infectious Diseases – Tuberculosis*. Centers for Disease Control and Prevention. www2.ncid.cdc.gov/travel/yb/utils/ybGet.asp?section=dis&obj=tb.htm.

Chakraborty, A. (2003) Diseases of elephants

(*Elephas maximus*) in India. A review. *Indian Wildlife Year Book* **2:** 74-82.

Chandrasekharan, K., Radhakrishnan, K., Cheeran, J.V., Nair, K.N.M. & Prabhakaran, T. (1995) Review of the incidence, etiology and control of common diseases of Asian elephants with special reference to Kerala. In: *A Week with Elephants. Proceedings of the International Seminar on Asian Elephants*. Daniel, J.C. (ed.) Bombay Natural History Society, Oxford University Press, Bombay, India. pp 439-449.

Chandrasekharan, K. (2002) Specific diseases of Asian elephants. *Journal of Indian Veterinary Association Kerala* **7(3):** 31-34.

Dalovisio, J.R., Montenegro-James, S., Kemmerly, S.A., Genre, C.F., Chambers, R., Pankey, G.A., Failla, D.M., Haydel,K.G., Hutchinson,L., Lindley,M.F., Praba,A., Eisenach, K.D. & Cooper, E.S. (1996) Comparison of the amplified *Mycobacterium tuberculosis* (MTB) direct test, aplicor MTB PCR and IS6, 110-PCR for detection of MTB in respiratory specimens. *Clinical Infectious Diseases* **23:** 1099-1106.

Davis, M. (2001) *Mycobacterium tuberculosis* risk for elephant handlers and veterinarians. *Applied Occupational and Environmental Hygiene* **16:** 350-353.

Dumonxeaux, G.A. & Mikota, S. (2006) Tuberculosis treatment protocols and complications for elephants. In: *Proceedings of the International Elephant Conservation and Research Symposium*. Copenhangen, Denmark. pp 84-85.

Gavier-Widen, D., Hard Af Segerstad, C., Roken, B., Moller, T., Bolske, G. & Sternberg, S. (2002) *Mycobacterium tuberculosis* infection in Asian elephants (*Elephas maximus*) in Sweden. *European Association of Zoo and Wildlife Veterinarians 4th Scientific Meeting*.

Iyer, A.K. (1937) Veterinary science in India, ancient and modern with special reference to tuberculosis. *Agric. Livest. India* **7:** 718-724.

Lacasse, C, Terio, K., Kinsel, M.J.,Farina, L.L., Travis, D.A., Greenwald, R., Lyashchenko, K.P., Miller, M. & Gamble, K.C. (2007) Two cases of atypical mycobacteriosis caused by *Mycobacterium szulgai* associated with mortality in captive African elephants (*Loxodonta africana*). *Journal of Zoo and Wildlife Medicine* **38:** 101-107.

Larsen, R.S., Salman, M.D., Mikota, S.K., Isaza, R., Montali, R.J. & Triantis, J. (2000) Evaluation of a multiple-antigen enzyme-linked immunosorbent assay for detection of *Mycobacterium tuberculosis* infection in captive elephants. *Journal of Zoo and Wildlife Medicine* **31:** 291-302.

Lewerin, S.S., Olsson, S.L., Eld, K., Roken, B., Ghebremichael, S., Koivula, T., Kallenius, G. & Bolske, G. (2005) Outbreak of *Mycobacterium tuberculosis* infection among captive Asian elephants in a Swedish zoo. *The Veterinary Record* **156:** 171-175.

Lyashchenko, K., Singh, M., Colangeli, R. & Gennaro, M.L. (2000) A multi-antigen print immunoassay for the development of serological diagnosis of infectious disease. *Journal of Immunological Methods* **242:** 91-100.

Lyashchenko, K.P., Greenwald, R., Esfandiari, J., Olsen, J.H., Ball, R., Dumonceaux, G., Dunker, F., Buckley, C., Richard, M., Murray, S., Payeur, J.B., Andersen, P., Pollock, J.M., Mikota, S., Miller, M., Sofranko, D. & Waters, W.R. (2006) Tuberculosis in elephants: antibody responses to defined antigens of *Mycobacterium tuberculosis*, potential for early diagnosis, and monitoring of treatment. *Clinical and Vaccine Immunology* **13:** 722-732.

Maslow, J. 1997. Tuberculosis and other mycobacteria as zoonoses. In: *Proceedings American Association of Zoo Veterinarians*. Houston, Texas. pp 110-115.

Maslow, J.N., Mikota, S.K., Zhu, M., Riddle, H. & Peloquin, C.A. (2005) Pharmacokinetics of ethambutol (EMB) in elephants. *Journal of Vet. Pharmacology and Therapeutics* **28:** 321-323.

Maslow, J.N., Mikota, S.K., Zhu, M., Isaza, R., Peddie, L.R., Dunker, F., Peddie, J., Riddle, H., and Peloquin, CA. 2005. 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* **28:** 1-7.

McGaughey, C.A. (1961) Diseases of elephants. Part 3. *Ceylon Veterinary Journal* **9:** 94-98.

Michalak, K., Austin, C., Diesel, S., Bacon, M.J., Zimmerman, P. & Maslow, J.N. (1998) *Mycobacterium tuberculosis* infection as a zoonotic disease: transmission between humans and elephants. *Emerging Infectious Diseases* **4:** 283-287.

Michel A.L., Bengis R.G., Keet D.F. Hofmeyr, M., de Klerk, L.M., Cross, P.C., Jolles, A.E., Cooper, D., Whyte, I.J., Buss, P. & Godfroid, J. (2006) Wildlife tuberculosis in South African conservation areas: Implications and challenges. *Veterinary Microbiology* **112:** 91-100.

Mikota, S.K., Sargent, E.L. & Ranglack, G.S. (1994) *Medical Management of the Elephant*. Indira Publishing House, West Bloomfield, MI. pp 123-128.

Mikota, S.K., Larsen, R.S. & Montali, R.J. (2000) Tuberculosis in elephants in North America. *Zoo Biology* **19:** 393-403.

Mikota, S.K., Peddie, L., Peddie, J., Isaza, R., Dunker, F., West, G., Lindsay, W., Larsen, R.S., Salman, M.D., Chatterjee, D., Payeur, J., Whipple ,D., Thoen, C., Davis, D.S., Sedgwick, C., Montali, R., Ziccardi, M. & Maslow, J. (2001) Epidemiology and diagnosis of *Mycobacterium tuberculosis* in captive Asian elephants (*Elephas maximus*). *Journal of Zoo and Wildlife Medicine* **32:** 1-16.

Mikota, S.K., Dumonceaux, G., Miller, M., Gairhe, K., Giri, K., Cheeran, J.V., Abraham, D., Lyashchenko, K., Larsen, R.S., Payeur, J., Waters, W.R. & Kaufman, G. (2006a) Tuberculosis in Elephants: An Update on Diagnosis and Treatment; Implications for Control in Range Countries. In: *Proceedings of the International Elephant Conservation and Research Symposium*. Copenhangen, Denmark. pp 110-118.

Mikota, S.K., Miller, M., Dumonceaux, G., Giri, K., Gairhe, K., Hamilton, K., Paudel, S. & Vincent, B. (2006b) Elephant tuberculosis diagnosis: implications for elephant management in Asian range countries. In: *Proceedings American Assoc iation of Zoo Veterinarians*. pp 142-143.

Mikota, S.K. (2008) Tuberculosis in elephants. In: *Zoo and Wild Animal Medicine, Current Therapy, 6th Edition.* Fowler, M.E. & Miller, R.E. (eds.) Saunders/Elsevier, St. Louis. pp 355-364.

Moller, T., Roken, B., Petersson, L., Vitaud, C. & Lyashchenko, K. (2005) Preliminary results of a new serological test for detection of TB-infection (*Mycobacterium tuberculosis*) in elephants (*Elephas maximus* and *Loxodonta africanum*) - Swedish Case studies. *Verh. ber. Erkrg. Zootiere* **42:** 173-181.

Moller, T., Roken, B O., Sternberg Lewerin, S. & Lyashchenko, K. (2006) The elephant Rapid Test (RT) - the future diagnostic test for TB (*M. tuberculosis*) in elephants? In: *Proceedings of the International Elephant Conservation and Research Symposium*. Copenhagen Zoo. pp 119-124.

Montali, R.J., Mikota, S.K. & Cheng, L.I. (2001) *Mycobacterium tuberculosis* in zoo and wildlife species. *Revue Scientifique et Technique Office International des Epizooties* **20:** 291-303.

Montali, R.J. (2006) Postmortem diagnostics. In: Fowler, M.E. & Mikota, S.K.(eds). *Biology, Medicine, and Surgery of the Elephant*. Blackwell Publishing, Ames, IA. pp 199-209.

Narayanan, R.S. (1925) A case of tuberculosis in an elephant. *Journal of Comparative Pathology* **38:** 96-97.

Nuermberger, E., Bishai, W.R. & Grosset, J.H. (2004) Latent tuberculosis infection. *Seminars*

in Respiratory and Critical Care Medicine **25:** 317-336.

Oh, P., Granich, R., Scott, J., Sun, B., Joseph, M., Stringfield, C., Thisdell, S., Staley, J., Workman-Malcolm, D., Borenstein, L., Lehnkering, E., Ryan, P., Soukup, J., Nitta, A. & Flood, J. (2002) Human exposure following *Mycobacterium tuberculosis* infection of multiple animal species in a Metropolitan Zoo. *Emerging Infectious Diseases* **8:** 1290-1293.

Payeur, J.B., Jarnagin, J.L., Marquardt, J.G. & Whipple, D.L. (2002) Mycobacterial isolations in captive elephants in the United States. *Annals of the New York Acad. of Sciences* **969:** 256-258.

Peloquin, C.A. (2002) Therapeutic drug monitoring in the treatment of tuberculosis. *Drugs* **62:** 2169-2183.

Pinto, M.R.M., Jainudeen, M.R. & Panabokke, R.G. (1973) Tuberculosis in a domesticated Asiatic elephant *Elephas maximus*. *Veterinary Record* **93:** 662-664.

Rahman, T. (2003) Infectious and non-infectious disease of elephants. In: *Healthcare, Breeding and Management of Asian Elephants*. Das, D. (ed.) New Delhi Project Elephant. Govt. of India. pp 108-118.

Ratanakorn, P. (2001) Elephant health problems and management in Cambodia, Lao and Thailand. A research update on elephants and rhinos. In: *Proceedings of the International Elephant and Rhino Research Symposium*. Vienna. pp 111-114.

Rothschild, B.M., Helbling, M. & Laub, R. (2006) Hyperdisease in the late Pleistocene: Validation of an early 20TH century hypothesis. *Naturwissenchaften* **93:** 557-564.

Ryan, C.P. (1997) Tuberculosis in circus elephants. In: *Pulse Southern California Veterinary Medical Assoc.* (January): 8.

Seneviratna, P., Wettimuny, S.G. & Seneviratna, D. (1966) Fatal tuberculosis pneumonia in an elephant. *Veterinary Medicine Small Animal Clinician* **60:** 129-132.

WHO (2007) *Tuberculosis. Fact Sheet N°104*. World Health Organization. www.who.int/mediacentre/factsheets/fs104/en/.

WHO (2008) *Global Tuberculosis Control - Surveillance, Planning, Financing*. World Health Organization. www.who.int/tb/publications/global_report/en/index.html.

Zhu, M., Maslow, J.N., Mikota, S.K., Isaza, R., Dunker, F. & Peloquin, C.A. (2005) Population pharmacokinetics of pyrazinamide in elephants. *Journal of Veterinary Pharmacology and Therapeutics* **28:** 403-409.

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Herd near Kaudulla National Park, Sri Lanka (May 2008)
Photo by Jennifer Pastorini