Tuberculosis in Elephants in North America

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Within the past 4 years, tuberculosis (TB) has emerged as a disease of concern in elephants. The population of elephants in North America is declining [Wiese, 1997], and transmissible diseases such as TB may exacerbate this trend. Guidelines for the Control of Tuberculosis in Elephants, which require the screening of all elephants for TB, were instituted in 1997 [USDA, 1997; 2000]. Between August 1996 and May 2000, *Mycobacterium tuberculosis* was isolated from 18 of 539 elephants in North America, indicating an estimated prevalence of 3.3%. Isolation of the TB orga nism by culture is the currently recommended test to establish a diagnosis of TB; however, culture requires 8 weeks. Further research is essential to validate other diagnostic tests and treatment protocols. Zoo Biol 19:393–403, 2000. © 2000 Wiley-Liss, Inc.

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INTRODUCTION

Tuberculosis (TB) was first described in elephants more than 2,000 years ago by ancient Ayurvedic physicians in Ceylon [Iyer, 1937; McGaughey, 1961]. In recent times, a case in London [Garrod, 1875] was followed by sporadic reports throughout the twentieth century [Narayanan, 1925; Baldrey, 1930; Gutter, 1981; Saunders, 1983; Chandrasekharan, et al., 1995]. Although it is thought that elephants are susceptible to *Mycobacterium bovis* [Dannenberg, 1978; Schmidt, 1986], *Mycobacterium tuberculosis* (*M. tuberculosis*) has been identified as the causative agent in all cases in which bacteria have been isolated. To date, most reported cases of TB have occurred in captive Asian elephants (*Elephas maximus*). Two suspected cases in Af-

rican elephants (*Loxodonta africana*) in Uganda and Israel were not confirmed by culture [Woodford, 1982; Gorovitz, 1962]. *M. tuberculosis* was isolated from an African elephant in France [Urbain, 1938]. In a retrospective medical study of 379 elephants in North American zoos, eight elephants died of TB between 1908 and 1994 [Mikota et al., 1994]. It is likely that this figure would have been higher had privately owned elephants been included in the survey.

RECENT CASES (1996–2000)

Between August 1996 and June 2000, samples from 539 elephants were submitted to the National Veterinary Services Laboratory (NVSL, Ames, IA) for mycobacterial culture [J. Payeur, personal communication]. Seventeen elephants from eight herds in Illinois, California, Arkansas, Missouri, and Florida were diagnosed with TB. At the time of diagnosis, three elephants resided in American Zoo and Aquarium Association accredited zoos and 14 in private facilities. There had been known previous contact between elephants in five of the herds. Three elephants demonstrated clinical signs that could be caused by TB. *M. tuberculosis* was isolated from 12 elephants pre-mortem and five elephants post-mortem. Restriction fragment length polymorphism was performed on *M. tuberculosis* isolates from 11 elephants. Five distinct *M. tuberculosis* strains were identified by this analysis [D.L. Whipple, 1997; personal communication].

Based on the 539 elephants in the NVSL database and the 532 elephants identified by the North American Regional Studbook keepers [M. Keele and D. Olson, personal communication], the prevalence of TB in elephants in North America is estimated to be 3.3%.

CLINICAL SIGNS

Confirmed cases of TB in elephants have typically been identified on post-mortem examination as ante-mortem signs are frequently absent. Chronic weight loss, anorexia. and weakness may occur [McGaughey, 1961; Gutter, 1981; Saunders, 1983], and dyspnea and coughing are sometimes observed [Seneviratna et al., 1966; Pinto et al., 1973]. Exercise intolerance is most likely to be observed in working animals [K.U. Mar, personal communication]. In Ceylon, ancient Ayurvedic elephant physicians regarded ventral edema as a sign of incurable lung disease [Pinto et al., 1973]. More recently, ventral edema has been observed in some TB-infected elephants; however, it may have been caused by concurrent congestive heart failure, anemia, or other medical conditions [Seneviratna et al., 1966; Pinto et al., 1973].

DIAGNOSIS

Several techniques have been used to diagnose mammalian TB. Methods such as culture, acid fast smears, fluorescent smears, and nucleic acid amplification techniques directly detect the bacterial organism. Indirect methods such as serological assays, the gamma-interferon test (GIT), and the intra-dermal tuberculin test detect antigen-antibody or cellular reactivity to mycobacterial antigen [Mikota and Maslow, 1997].

In humans and domestic animals, the intra-dermal tuberculin skin test is the primary screening method to detect infection with *M. tuberculosis* or *M. bovis*. The

intra-dermal tuberculin test is the official ante-mortem test in cattle, bison, and deer and is incorporated into the U.S. Department of Agriculture (USDA) eradication guidelines [Essey and Davis, 1997; USDA, 1994; 1999]. The blood TB test (BTB), enzyme-linked immunosorbent assay (ELISA), and GIT have been used to diagnose TB infection in a limited number of species [Griffin and Cross, 1989; Rothel et al., 1992; Gaborick et al., 1996]. These indirect methods have not been validated in most non-domestic species nor have they been uniformly administered or consistently interpreted [Montali and Hirschel, 1990]. The limitations of applying such diagnostic tests to non-domestic species have been discussed [Hietala and Gardner, 1999].

Intra-dermal tuberculin testing is used in conjunction with slaughter surveillance to control TB in domestic cattle [Thompson et al., 1998]. Obviously, this method should not be applied to species such as the elephant. The current gold standard for the ante-mortem diagnosis of *M. tuberculosis* infection in elephants is mycobacterial culture of respiratory secretions obtained by trunk "wash" [USDA, 1997; 2000]. Elephants must be conditioned to permit the trunk wash procedure, which consists of the instillation of 60 mL of sterile saline into one or both nostrils, application of a 1-gal plastic bag over the end of the trunk, elevation of the trunk to distribute the saline, and collection of a forcibly exhaled sample into the bag [Isaza and Ketz, 1999]. Because the TB organism can be shed intermittently, three samples are collected on separate days.

To date, intra-dermal and serologic tests have frequently shown poor correlation with mycobacterial culture results in elephants [Montali et al., 1998]. One elephant that died from disseminated pulmonary *M. tuberculosis* infection had negative results when tested with human and bovine purified protein derivative (BPPD) tuberculins [Saunders, 1983]. Furthermore, four of five *M. tuberculosis* culture-positive elephants diagnosed since 1996 have demonstrated negative intra-dermal tuberculin responses to BPPD or balanced tuberculins. Eight of 30 *M. tuberculosis* culture-negative elephants had suspect tuberculin responses [Mikota, 1999].

Nucleic acid amplification techniques (NAAT) such as PCR detect mycobacteria by amplification of DNA or RNA [de Wit et al., 1990; Clarridge et al., 1993; Liébana et al., 1995; Roberts et al., 1996]. Advantages of NAAT include rapid turnaround time (hours) and the capability of detecting low numbers of organisms. These techniques have been reported to have high specificity for *M. tuberculosis complex* organisms (*M. tuberculosis, M. bovis, M. africanum, M. microti*), but mycobacterial species cannot be differentiated. Since both live and dead organisms are detected, NAAT is of limited value in monitoring response to therapy.

There are at present two amplification assays that are approved by the U.S. Food and Drug Administration for commercial use in human clinical and public health laboratories: the Amplicor MTB PCR (Roche Molecular Systems, Branchburg, NJ) and the Amplified *M. tuberculosis* Direct Test (MTD) (Gen-Probe, San Diego, CA). These assays have demonstrated specificities of ≥95%, but they have been less sensitive than culture for diagnosis of *M. tuberculosis* infection in humans [Dalovisio et al., 1996]. Validation has not yet been reported in elephants. Between August 1996 and August 1999, the NVSL performed the MTD on 612 elephant samples. The MTD correctly diagnosed six of nine culture-positive samples and 559 of 581 culture-negative samples. Results of 23 samples were inconclusive [J. Payeur, personal communication]. Using culture as the gold standard, the MTD demonstrated a sensitivity of 66.6% and a speci-

ficity of 96.2%. Validation of this test is still needed, and, at this time, the MTD and other NAATs are considered ancillary tests for detecting TB in elephants.

Serologic testing for TB has been performed using ELISA techniques with a non-species–specific protein A that is labeled with horseradish peroxidase. Early investigation of this technique in a herd of five elephants, which included one culture-positive animal, was described by Thoen and colleagues [1980]. The antigens used were heat-killed cells of *M. bovis,* BPPD, and purified protein derivative of *M. avium* (APPD). Two tuberculin-test positive elephants showed substantial seroreactivity to heat-killed *M. bovis* and BPPD, whereas two of three tuberculin-test negative animals showed seroreactivity only to heat-killed *M. bovis.*

Between 1997 and 1998, during the evaluation of a herd of four culture-negative elephants, it was proposed that tuberculin exposure may influence serologic and BTB analysis [Montali et al., 1998]. The antigens used in this serologic investigation were BPPD, *M. tuberculosis* culture filtrate protein, *M. avium* culture filtrate protein, APPD, and a lipoarabinomannan antigen (LAM) derived from *M. tuberculosis.* Elephants showed minimal to zero reactivity to these antigens before intra-dermal tuberculin injection, but dramatic increases in seroreactivity after injection. Elephants are constantly exposed to saprophytic *Mycobacterium* species and can be colonized by non-tuberculous mycobacteria (as well as *M. avium*) due to their behavior of bathing and dusting using their trunks. These organisms do not appear to cause clinical disease, but it has been hypothesized that such exposure accounts for these seemingly non-specific reactions to mycobacterial antigens [Montali et al., 1998].

Recently, a multiple-antigen ELISA was evaluated for its ability to detect *M. tuberculosis* infection in captive elephants [Larsen et al., 2000]. Serum samples were collected from 32 Asian and 15 African elephants, and a panel of six antigens was used to determine seroreactivity. The antigens included *M. bovis* culture filtrate (CF), PPD, *M. bovis* modified protein 70, two LAM antigens from strains of *M. tuberculosis*, and APPD. Discriminant analysis was used to determine the linear combination of antigens that accurately predicted the true infection status of the most animals, and the resulting classification functions were used to calculate the percentage of animals that were correctly classified. Of 47 elephants, seven of the Asians were infected (culture-positive); 25 Asians and 15 Africans were considered non-infected (culture-negative). Criteria for elephants designated as non-infected were: 1) trunk washes within 4 months of serum sampling that were negative for mycobacterial culture of *M. tuberculosis* or *M. bovis*; 2) no contact with elephants or other animals that had been diagnosed with *M. tuberculosis* of *M. bovis* within the past 5 years; 3) no intra-dermal tuberculin testing within the 6 months before sampling; and 4) no travel outside the institution in the previous 5 years. The specificity and sensitivity of the multiple-antigen ELISA, with 95% confidence intervals, were 100% (91.9–100%) and 100% (54.4–00%), respectively. The limitations inherent to this study suggest that much additional research is needed regarding the use of this ELISA; however, the results also indicate that this multiple-antigen ELISA may be a good screening test for elephants [Larsen et al., 2000].

PATHOLOGIC FINDINGS

The major pathology in elephants infected with *M. tuberculosis* infections occurs primarily in the lungs and thoracic lymph nodes with lesser involvement of

extra-thoracic sites [Seneviratna et al., 1966; Pinto et al., 1973, Gutter 1981; Saunders 1983; Michalak et al., 1998; R.J. Montali, unpublished observations, 1999]. As in other animals, tubercular lesions in elephants appear to vary with the staging of the disease. In the less extensive cases, firm granulomatous nodules, sometimes with caseous foci, are noted in the bronchial lymph nodes and pulmonary tissue. Elephants with extensive involvement of both lungs (≥66%) usually die with severe caseo-calcareous and cavitating lesions. These often result in large pulmonary abscesses from which *M. tuberculosis* and opportunistic bacteria such as *Pseudomonas aeruginosa* have been isolated. Tenacious, mucopurulent, bronchial plugs are also common in advanced TB; bronchial and other thoracic lymph nodes are markedly enlarged and usually show a proliferative response with less caseation than the pulmonary lesions.

The pathologic descriptions "caseous" and "mucopurulent" are based on post-mortem observations of the lungs and upper respiratory tract. At post-mortem, the severity of the disease is determined and a time frame ascribed. Active, early lesions, which conceivably can occur within weeks to months, are usually localized and limited in size and scope. Advanced lesions may involve major portions of one or both lung lobes and become mineralized over a period of months to years. Both, however, may have areas that are caseous or mucopurulent.

Characteristic histologic findings include epithelioid granulomas with some giant cell formation in the earlier lymph node and pulmonary lesions and extensive caseous and pyogranulomatous pneumonia in the advanced forms. Though sparse, acid-fast bacilli are more easily found in central areas of caseation in the lungs but are typically rare in the lymph nodes.

Bronchial and tracheal tuberculous plaques and caseous and mucopurulent exudate in the nasal passages have been noted in both the early and late stages of TB, suggesting that the shedding of mycobacteria may occur at any stage of the disease. Less extensive tuberculous lesions were observed in the mesenteric lymph nodes, liver, kidneys, adrenals, and spleen in some of the more advanced cases. These lesions suggest that in disseminated cases shedding may occur by routes other than the respiratory system.

TREATMENT

There is little information in the literature regarding treatment of elephants for TB. In one report, an Asian elephant was treated prophylactically with isoniazid (INH) after a suspect intradermal tuberculin test [Devine et al., 1983]. Another suspected case of TB was treated with streptomycin administered intramuscularly on alternate days for 4 weeks [Chandrasekharan et al., 1995]. Current treatment protocols have been extrapolated from human treatment regimens [American Thoracic Society, 1994] and are still under investigation for efficacy in elephants.

Anti-TB drugs recently used in elephants includes INH, pyrazinamide (PZA), rifampin (RIF), and ethambutol (ETH). These drugs have been administered to elephants in food, by direct oral administration, and rectally. Oral delivery has been challenging, as many elephants refuse oral medications. Direct oral administration can be achieved in some elephants by conditioning the animals to accept a bite block and oral syringe [L. Peddie and J. Peddie, personal communication]. For other elephants, rectal drug administration techniques (including suppositories) have been developed.

Blood levels of INH, consistent with human therapeutic values, can be achieved in elephants by direct oral or rectal administration. Blood levels of RIF can be achieved orally, but not rectally [Dunker and Rudovsky, 1998]. PZA appears to be absorbed by either route [S.K. Mikota, unpublished data]. When anti-TB drugs are administered in food, blood levels are variable and this route of administration is not recommended. Anti-TB drug doses for individual elephants should be determined by measuring blood-level response. Elephants should be weighed before and throughout treatment.

The current recommended treatment for known infected elephants consists of INH and RIF daily for 2 months, then every other day for 10 months. A third drug, such as PZA, is given daily for the first 2 months of treatment. As a starting dose, INH can be given orally or rectally at a dose of 2.5–5.0 mg/kg. Although humans typically achieve a blood level of 3–5 µg/mL of INH at 2 hours [C.A. Peloquin, personal communication], some elephants became ill when their blood levels were in this range. An INH blood level of 1–2 µg/mL is recommended for elephants.

RIF can be initiated orally at a dose of 7.5–10.0 mg/kg orally. Human 2-hour blood levels for this drug are 8–24 µg/mL. PZA can be initiated at a dose of 25–35 mg/kg orally or rectally. Human 2-hour levels for PZA are 20–60 µg/mL. Supplementation with vitamin B6 (pyridoxine) at a daily dose of 1 mg/kg is recommended to prevent possible peripheral neuropathy, a condition that has been associated with INH therapy in humans [Goldman and Braman, 1972].

Side effects of treatment may include anorexia, lethargy, and colitis. Leukopenia was observed in one elephant receiving INH and RIF; the condition resolved after treatment was temporarily stopped and dosages adjusted [L. Peddie and J. Peddie, personal communication]. Elevations of liver enzymes have been observed in association with INH toxicity in humans and elephants.

Of 11 living *M. tuberculosis* culture-positive elephants, six are currently receiving anti-TB drugs including one elephant that is undergoing a second course of treatment. Five elephants that completed treatment in December 1997, June 1998, December 1998, October 1999, and April 2000, are presently culture negative. All culture-positive elephants ceased shedding organisms shortly after treatment was initiated and remained culture-negative during the treatment period.

GUIDELINES FOR THE CONTROL OF TB IN ELEPHANTS

In 1996, in response to the TB-related deaths of two privately owned elephants, an Elephant Tuberculosis Advisory Panel was formed. This panel, which was composed of USDA and zoo veterinarians, cooperated with the National Tuberculosis Working Group for Zoo and Wildlife Species to develop *Guidelines for the Control of Tuberculosis in Elephants* [USDA, 1997; 2000; www.aphis.usda.gov/ac/acindex.html]. These guidelines specify criteria for the testing, surveillance, and treatment of elephants for TB. In January 1998 and January 2000, they were distributed by the Animal Care Division of the USDA Animal Plant Health Inspection Service (USDA-APHIS) to all licensed elephant exhibitors regulated by the Animal Welfare Act. The guidelines require annual testing (three trunk cultures) of all elephants and strongly recommend submission of samples for ancillary diagnostic tests.

According to the guidelines, elephants are placed in one of four groups based on culture results and exposure history. Group A elephants have negative culture results and no known exposure to a culture-positive animal in the previous 5 years. These animals are cultured annually and have no travel restrictions while they remain culture negative for *M. tuberculosis*. Culture-negative elephants exposed to a culture-positive animal 1–5 years previously (group B) are cultured quarterly and have no travel restrictions. Culture-negative elephants exposed to a culture-positive animal within the previous 12 months (group C) may be monitored by culture (three-sample method), every other month for 1 year, with no travel permitted or, alternatively, may be treated, with travel permitted after 2 months if cultures remain negative. Culture-positive elephants (group D) are not permitted to travel until at least 6 months of treatment have been completed and two negative cultures have been demonstrated. Figure 1 illustrates the sequence of events for each of the four groups.

A thorough post-mortem examination should be performed on all elephants that die or are euthanized. Lungs and lymph should be closely examined for evidence of TB. Cultures for TB should be submitted on all elephants even if gross lesions are absent. A necropsy protocol for elephants may be accessed at the above website.

IMPLICATIONS FOR HUMAN HEALTH

After the diagnosis of TB in the Illinois herd, all personnel were tuberculin tested by the Illinois Department of Health. Of 22 handlers, 11 were tuberculin test positive. Eight of the 11 were positive on the initial test, indicating the possibility of prior exposure; three individuals converted during the investigation. One handler had culture-positive TB. The isolate from this individual matched that of the Illinois elephants [Michalak et al., 1998]. The original source of infection (for both elephants and humans) is unknown.

The apparent low incidence of TB in African elephants and the absence of reports of TB in free-ranging elephants suggest that this is primarily a disease of humans and that elephants are accidental hosts. Nonetheless, *M. tuberculosis* can be transmitted between elephants and humans and must be considered zoonotic [Maslow, 1997; Michalak et al., 1998]. Humans are most likely to contract TB when they have prolonged contact with infected individuals. It is likely that this is also the case with elephants, suggesting that handlers with close, daily contact with infected animals are at greatest risk. Elephant handlers and other personnel in contact with elephants should be tested for TB annually following established human testing protocols. All new employees should be tested before contact with elephants and anyone with active TB should not have contact with elephants. Some zoos have developed elephant/people interaction protocols to limit direct visitor contact with elephants, a practice that should be encouraged [Montali, 1999].

RESEARCH ISSUES

The ante-mortem diagnosis of TB in elephants continues to be problematic. Although identification of *M. tuberculosis* definitively establishes the presence of infection, failure to isolate the organism does not rule out infection. Mycobacteria are slow-growing organisms and culture typically requires 8 weeks. Clearly, better

All Elephants: 3 Trunk Cultures

Group A

Negative culture
No exposure past
5 years

↓

Culture annually
Ancillary tests
recommended

↓

Send annual report
to AAZV

Group B

Negative culture
No clinical signs
Exposure to culture (+)
animal 1-5 years ago

↓

Culture quarterly for 1 year
Ancillary tests recommended
No travel or treatment restriction

↓

All cultures negative → Culture annually → Send annual report to AAZV

Positive culture → See group D

Group C

Negative culture
Exposure to culture (+)
animal within last 12
months +/- clinical sign

↓

Reculture 3 samples
over one week

↓

Culture positive → See group D

Culture negative → Ancillary tests recommended

Option 1 → Treat for 6 months (see protocol) No travel for 2 months, can travel after 2 months with negative culture and concurrent treatment → Monitor CBS/SMAC monthly during treatment Collect cultures quarterly for 18 months →
- Culture negative → Culture annually → Send annual report to AAZV
- Culture positive → Go to group D

Option 2 → Culture every other month for 1 year. No travel for 12 months. →
- Cultures negative → Culture annually → Send annual report to AAZV
- Cultures positive → Go to group D

Group D

Positive culture

↓

Perform ancillary tests
- Acid fast smear
- ELISA
- Nucleic acid amplification
- Gamma - interferon

↓

Treat for 12 months (see protocol)
No travel until 6 months of treatment
complete and 2 consecutive negative
cultures. Monitor CBC/SMAC monthly
Take 3 cultures monthly during
treatment

↓

Culture monthly for 6 months post-
treatment, then culture every other
month for 6 months

↓

- All cultures negative → Culture annually → Send annual report to AAZV
- Cultures positive → Retreat

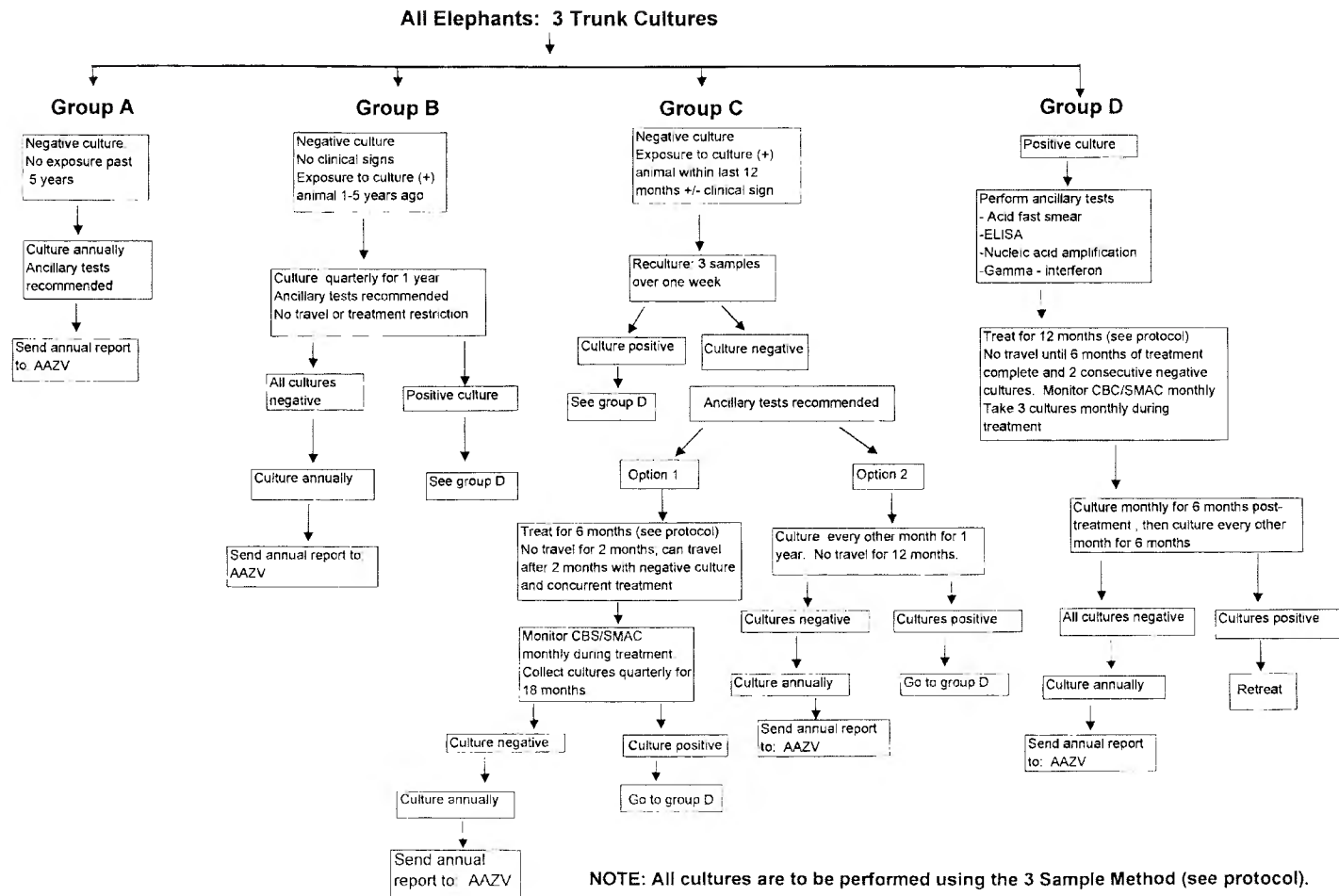**NOTE: All cultures are to be performed using the 3 Sample Method (see protocol).**

Fig. 1. Elephant management groups for TB surveillance [from USDA, 2000]. (For protocol, see Guidelines for the Control of Tuberculosis in Elephants [USDA, 2000] www.aphis.usda.gov/ac/acindex.html).

diagnostic tests are needed. Preliminary ELISA results are promising, but many more samples (both culture positive and negative) must be analyzed before this test (or any other serologic test) can be validated. Institutions holding elephants are strongly encouraged to submit samples for ancillary diagnostic tests so that valuable research data may be collected.

Although there is optimism that infected elephants in the North American population have been successfully treated, only long-term monitoring will confirm this. Pharmacokinetic studies are needed to further evaluate anti-TB drugs and to validate therapeutic protocols. It is essential that elephants that undergo treatment be observed for possible side effects. Blood levels of anti-TB drugs must be documented and correlated with treatment outcome.

Information on the pathophysiology and staging of TB in elephants is needed. A necropsy should be performed on all elephants that die and a thorough search for TB lesions should be conducted, even if the disease is not suspected. The elephant necropsy protocol (available on the USDA website) outlines appropriate samples to submit for laboratory evaluation. In cases of euthanasia, a diagnostic workup for TB (including ancillary tests as outlined in the guidelines) should be performed pre-mortem so that results can be correlated with postmortem findings.

A reporting mechanism has been established whereby annual culture and ancillary test results are submitted to the American Association of Zoo Veterinarians for tabulation. Compliance has been poor for this critically needed information. We encourage the cooperation of the zoo community to comply with this reporting mechanism so that we may further our understanding of TB in elephants.

CONCLUSIONS

1. Reported cases of TB in elephants have been caused by *M. tuberculosis* (the agent of human TB). Although TB has been reported more frequently in Asian elephants, it is unknown whether there is a true species predilection.

2. Isolation of *M. avium* and non-tuberculous mycobacteria from elephant trunk wash samples is common, but these organisms have not been associated with clinical disease.

3. Isolation of *M. tuberculosis* is currently the only definitive test to diagnose TB in elephants, although ancillary tests such as NAAT and ELISA may be useful.

4. The intra-dermal tuberculin test is unreliable for diagnosing TB in elephants.

5. It is possible to deliver dosages of anti-TB drugs that achieve blood levels consistent with therapeutic levels in humans; however, the long-term efficacy of current treatment protocols remains to be documented.

6. Shedding of TB organisms generally ceases when elephants receive adequate levels of anti-TB drugs.

7. Transmission of TB between an elephant and a human has been reported. Handlers in close daily contact with infected elephants are at greatest risk.

8. Elephants may be at risk of contracting TB from infected humans. Handlers should undergo periodic TB screening to minimize risks to elephant health.

9. Complete post-mortem examination should be performed on all elephants that die. A thorough search for TB lesions should be conducted even if TB is not suspected.

10. Zoos are encouraged to establish protocols for elephant-visitor interactions.

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