

EPIDEMIOLOGY AND DIAGNOSIS OF *MYCOBACTERIUM TUBERCULOSIS* IN CAPTIVE ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)

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Abstract: The deaths of two Asian elephants (*Elephas maximus*) in August 1996 led the United States Department of Agriculture to require the testing and treatment of elephants for tuberculosis. From August 1996 to September 1999, *Mycobacterium tuberculosis* infection was confirmed by culture in 12 of 118 elephants in six herds. Eight diagnoses were made antemortem on the basis of isolation of *M. tuberculosis* by culture of trunk wash samples; the remainder (including the initial two) were diagnosed postmortem. We present the case histories, epidemiologic characteristics, diagnostic test results, and therapeutic plans from these six herds. The intradermal tuberculin test, enzyme-linked immunosorbent assay serology, the blood tuberculosis test, and nucleic acid amplification and culture are compared as methods to diagnose *M. tuberculosis* infection in elephants.

Key words: Elephant, *Elephas maximus*, *Loxodonta africana*, tuberculosis, *Mycobacterium tuberculosis*, tuberculin test.

INTRODUCTION

Tuberculosis (TB) has been recognized for over 2,000 yr as a disease of elephants.^{19,23} It has been most frequently reported in Asian elephants (*Elephas maximus*),^{2,5,13,17,28,29,31,32} Only one case in African elephants (*Loxodonta africana*) has been confirmed by culture,^{15,39,45} and all cultures in both species have involved *Mycobacterium tuberculosis*. A retrospective North American zoo study noted eight deaths from TB among 379 elephants. Four of these

cases occurred prior to 1941, and three were confirmed by culture.²⁵

Clinical signs of TB in elephants are comparable to those observed in humans and may include weight loss, anorexia, weakness, dyspnea, and coughing.^{17,23,29,31,32}

Definitive antemortem diagnostic techniques for TB in elephants have limitations. The intradermal (i.d.) tuberculin test, currently the accepted standard

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domestic animal screening test, has been validated only in domestic cattle, bison, and Cervidae and has been poorly correlated with culture results in several species.^{11,21,33} Other indirect methodologies, such as serologic assay and cellular responsiveness to mycobacterial antigens, also lack validation in nondomestic species. Radiographic thoracic evaluation is feasible only in young elephants.

Bison and Cervidae are the only nondomestic ungulates officially regulated under the National Cooperative State–Federal Bovine Tuberculosis Eradication Program, which is based on slaughter-surveillance to control *Mycobacterium bovis*.^{36–38} With elephants and other endangered species, treatment with intent toward cure is preferable to test and slaughter. Treatment is expensive, however, and may be associated with fatal, idiosyncratic effects in some species,⁴ so accurate diagnosis of both active and latent disease is of paramount importance.

Subsequent to the diagnoses of *M. tuberculosis* in two Asian elephants in 1996, an Elephant Tuberculosis Advisory Panel was formed. Through the efforts of this panel, the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services, and the National Tuberculosis Working Group for Zoo and Wildlife Species (formed in February 1997 and comprised of veterinarians from the American Association of Zoo Veterinarians and USDA), an elephant TB screening protocol was established.³⁵ This protocol, requiring the testing of elephants for TB, was sent to all licensed exhibitors in the United States in January 1998.

We review the presentation and clinical course of *M. tuberculosis* infection in 12 elephants from six affected herds. Culture results are compared with indirect methods of TB diagnosis including i.d. tuberculin testing, serologic responsiveness determined by enzyme-linked immunosorbent assay (ELISA) and lymphocyte stimulation, as well as direct testing by amplification to detect mycobacterial nucleic acids. We briefly describe treatment regimens and efficacy, although these findings will be reported and discussed elsewhere.

HISTORY OF AFFECTED HERDS

Herd A

On 3 August 1996, a 47-yr-old female Asian elephant (A1) died in California after anesthesia for a suspected dental problem. Weight loss had been noted since October 1995. At necropsy, disseminated, caseous, granulomatous lesions were observed in the cervical, tracheobronchial, and mediastinal lymph nodes. The serosal surfaces of the

liver, stomach, and peritoneum were covered with white, miliary, firm nodules. Numerous firm, white, nodular lesions were identified in the lungs, with less than 10% functional parenchymal mass remaining. Acid-fast bacteria were observed in 9 of 26 tissue smears. Culture samples from multiple tissues yielded *M. tuberculosis*.

Four elephants traveling with elephant A1 were en route to the home facility when a 26-yr-old female Asian elephant (A2) died on 6 August 1996. This animal was asymptomatic and in fair body condition. Postmortem examination revealed severe, multifocal, granulomatous pneumonia with numerous abscesses, caseation, and granulomatous lymphadenitis. Culture of lung tissue from this elephant also yielded *M. tuberculosis*.

In September 1996, a 37-yr-old male Asian elephant (A4) died suddenly, with a history of foot problems, weight loss, and inappetance preceding death. An enlarged heart and pericardial effusion were the most significant postmortem findings. Histologic examination showed no evidence of tuberculosis, and mycobacteria were not isolated. Eighteen elephants (A3, A5–A21) remained in the herd: one male Asian elephant (age 3 yr), 15 female Asian elephants (18–55 yr), and two female African elephants (16 and 18 yr).

Elephant facility A consisted of a large barn subdivided into small, medium, and large elephant holding areas. A small (3 m × 9 m) entry area was situated between the medium and large barns, and an enclosed exercise area was situated between the small and large barns. Ventilation ducts located in the ceiling were continuous between barns. Because of the northern location of the facility, the elephants were housed indoors for 3–4 mo during the winter.

Approximately 14 animals were kept in a line in the larger of the barns. Two to five animals were housed in the medium barn. The elephants were exercised together in a large outdoor paddock. The young Asian elephant male was housed in the small barn. Twelve elephants from herd A, including elephants A1 and A2 (which typically traveled together) and A3 (which traveled in a separate group), were leased to various circuses. Six animals did not travel.

Two cases of *M. tuberculosis* infection had been documented in herd A prior to this study period. The first case occurred in an 11-yr-old female Asian elephant that died in California in 1983.³¹ The second case was a 46-yr-old female Asian elephant that died in 1994. After the diagnosis of TB in elephants A1 and A2, the remaining 18 elephants were evaluated in October 1996 and December

1996. *Mycobacterium tuberculosis* was isolated from a trunk wash culture and a pharyngeal swab culture taken from a 46-yr-old female Asian elephant (A3). Treatment of the herd was initiated in December 1996; trunk cultures were collected quarterly during this period. Subsequent diagnostic evaluations were conducted in August 1997, January 1998, and December 1998.

Herd B

In March 1997, a 30-yr-old female Asian elephant (B1) died of severe fibrinonecrotic enterocolitis. Fecal cultures yielded *Salmonella typhimurium*, which was considered to represent the etiologic agent. Prior to death, the only significant clinical problem noted for this animal was chronic arthritis of the left hip. At necropsy, several small focal granulomas were found in the thoracic and abdominal cavities. Histology showed necrotizing, granulomatous lymphadenitis and multinucleated giant cells. Culture of these tissues yielded *M. tuberculosis*.

The remainder of the herd consisted of a 39-yr-old female Asian elephant (B2), a 12-yr-old male Asian elephant (B3), and two female African elephants, ages 32 and 37 yr (B4 and B5, respectively). Housing at facility B consisted of a large open-sided indoor barn area and a large dirt floor outdoor area. Within herd B, the four females were housed together and had extensive contact. Contact with the remaining male Asian was limited to interaction across a fence.

After the diagnosis of *M. tuberculosis* in elephant B1, trunk wash samples for culture were obtained from the remaining herd members in July 1997. Follow-up cultures were obtained every 1–2 mo for the next 6 mo and then quarterly. Prophylactic treatment of the herd was initiated. No other *M. tuberculosis*-infected animal was detected.

Herd C

Herd C consisted of a 29-yr-old female Asian elephant (C1), a 32-yr-old female Asian elephant (C2), and two female African elephants, ages 30 and 37 yr (C3 and C4, respectively). Elephant C1 joined herd C in March 1997; prior to that time it was in herd B. The two Asian elephants (C1 and C2) shared a barn and exercise area. The two African elephants (C3 and C4) were housed together in a separate area of the zoo, and there was no contact between the two groups.

Trunk wash samples for culture were obtained from elephants C1 and C2 in March 1997. *Mycobacterium tuberculosis* was isolated from elephant C1. Therapeutic treatment of elephant C1 and pro-

phylactic treatment of elephant C2 were initiated in May 1997. Follow-up cultures of elephants C1 and C2 were obtained on average every 4–6 wk through August 1999 and continue on a monthly schedule. The African elephants (C3 and C4) were initially cultured in January 1998. *Mycobacterium tuberculosis* was not isolated from elephant C2, C3, or C4.

Herd D

Trunk wash samples for culture were taken from this group of elephants in May 1997. Elephant A1 had died on the premises of herd D in August 1996, and elephant C1 (a culture-positive animal) had lived in this group. Herd D consisted of four female Asian elephants ranging in age from 29 to 32 yr (D1–D4). Three elephants (D5–D7) had been sent to other institutions for breeding. One, a 13-yr-old female Asian elephant (D5), left the herd in April 1996 and returned in September 1997. Elephants D6 and D7 left in February 1995 and returned in April 1998.

Housing at facility D was contained within an 18.3-m × 18.3-m concrete block structure with a well-drained concrete floor. Within that structure was one large (12 m × 12 m) and one smaller (6 m × 9 m) housing area. There was no shared air space connecting the two housing areas. Each area, when in use, was cleaned and sanitized daily. Each housing area had large doors on two sides that were left open throughout the day, affording cross ventilation and exposure to sunlight while the elephants were in one of two separate, 0.2-ha exercise corrals. The interior of one of the exercise corrals was arranged so the elephants could commingle or be separated from one another. The elephants were housed outdoors during daylight hours throughout the year.

Herd D was first tested in May 1997. *Mycobacterium tuberculosis* was isolated from elephants D1 and D2. Treatment was initiated in June 1997.

Herd E

Herd E consisted of one 10-yr-old male Asian elephant (E1), three female Asian elephants ages 24–40 yr (E2–E4), four male African elephants ages 15–19 yr, (E5–E8), and two female African elephants ages 15 and 20 yr (E9 and E10, respectively). The Asian and African herds were housed outside in large paddocks, 24 hr/day, for 7 mo of the year. The paddocks were separate but shared a common fence. For 5 mo, the herds were housed in two barns at night, again separated by species. The Asian elephant bull (E1) was housed in a separate barn and paddock with one of the Asian ele-

phant females. In previous years, some of the Asian elephant females had been housed with the African elephants.

In June 1998, trunk washes were performed on the 10 elephants. *Mycobacterium tuberculosis* was isolated from the male Asian elephant (E1); elephants E2–E10 were culture negative. Treatment of elephant E1 was initiated in October 1998. Serologic studies were conducted in October 1998 and March 1999. Cultures were obtained weekly on elephant E1 and every other month on elephants E2–E10.

Herd F

Herd F consisted of four groups of elephants (FB, FR, FT1, and FT2). The first, FB, was a breeding group comprised of 23 female Asian elephants ranging in age from 2 to 57 yr and eight male Asian elephants ranging in age from 1 mo to 32 yr (FB1–FB31). Group FR was a retirement group and included 12 female Asian elephants 31–57 yr, three male Asian elephants 12–54 yr, and one 52-yr-old female African elephant (FR1–FR16). Group FT1 included traveling elephants comprised of 14 female Asian elephants ages 15–47 yr (FT1–FT14), and FT2 was a traveling group of 12 female Asian elephants 29–49 yr and one 4-yr-old male Asian elephant (FT1–FT13).

Housing at the breeding facility consisted of one large barn (1,579 m²), several smaller barns, and a series of large outdoor paddocks. Housing at the retirement facility was similar. The two facilities were located in the same state but were separated geographically. The two traveling elephant groups were generally housed at separate fairgrounds facilities for 2 mo of the year. Screening for TB began in 1997. *Mycobacterium tuberculosis* was isolated from elephants FB1 and FB2 and elephants FR3 and FR4.

Contact between herds

There was extensive contact between five of the six herds. Elephant A1 died on the premises of facility D in August 1996. After her death, the barn used for treatment and necropsy was thoroughly cleaned with a mycobactericidal disinfectant. There had been no direct contact between elephant A1 and herd D elephants.

Elephant B1 resided in facility D from October 1991 through April 1994, when she moved to herd B. Elephants B3 and B4 had also been housed temporarily with herd D from fall 1993 through fall 1994. Otherwise, elephants B2, B3, and B4 were long-term members (>10 yr) of herd B. Elephant C1 had an extensive travel history. She joined herd

D in October 1991, moved to facility B in April 1994, was returned to facility D in November 1996, and then finally moved to facility C in March 1997. Elephant E1, born in 1988, was the son of elephant FB1. He was housed at facility D from September 1993 to February 1994 and again from March to May 1996. He was with herd A in early February 1996 and joined herd E in May 1996.

There was no contact between herd F and the other five herds. At the time of testing, the four groups of herd F did not intermingle. However, in previous years, there was extensive commingling among the four groups. Elephants of herd F primarily moved between the retirement group FR and group FT2, although movements also occurred between the breeding facility and both traveling groups. Seven of the elephants in the breeding group arrived from a separate facility in 1996. The FT1 and FT2 groups each included three elephants that were owned by another facility.

The histories of the six herds and the 12 *M. tuberculosis*-positive elephants are summarized in Tables 1 and 2.

METHODS

Cultures

Cultures were processed at six laboratories: the National Veterinary Services Laboratories (NVSL; Ames, Iowa 50011, USA; herds A, C, D, E, and F); the County of Orange Public Health Laboratory (Santa Ana, California 92706, USA; herds B, C, D, and F); the San Bernardino County Laboratory (San Bernardino, California 92412, USA; herd B); the San Francisco City and County Public Health Laboratory (San Francisco, California 94110, USA; herd C); the Arkansas Department of Health, Division of Public Health Laboratories (Little Rock, Arkansas 72205-3867, USA; herd E); and the Iowa State University (Ames, Iowa 50011, USA; herd F). Specimens consisted of trunk washes, trunk swabs, oral swabs, and feces.

Drug susceptibilities of isolates of *M. tuberculosis* were determined by the National Jewish Medical and Research Center (Denver, Colorado 80206, USA; elephant B1), the San Bernardino County Laboratory (elephant A1), the San Francisco City and County Public Health Laboratory (elephant C1), or the County of Orange Public Health Laboratory (elephants D2, FB1, FB2, and FR4). Susceptibility testing for isolates from elephants A2, A3, E1, and FR3 was not performed.

Trunk washes were obtained by one of two methods. The first method consisted of inserting a 14

Table 1. Mycobacterial culture results from six elephant herds.^a

Herd	No. elephants	No. Asian	No. African	Age range	No. elephants M.tb+ ^b	No. elephants M.tb-	No. samples	No. samples M.tb+	No. samples with atypical, unidentified mycobacteria or with <i>Nocardia</i>
A	21	19	2	3–55	3	18	380	4	4
B	5	3	2	12–39	1	4	62	1	14
C	4	2	2	29–37	1	3	160	7	18
D	7	7	0	13–32	2	5	411	4	93
E	10	4	6	10–40	1	9	199	2	117
F	75	74	1	<1–57	4	67	893	15	196
Total	122 ^c	109	13	<1–57	12	106	2,105 ^d	33	442

^a Culture source was trunk wash or swab except for herd A (69 fecal samples and 60 oral swabs) and herd E (three oral swabs and one semen sample).

^b M.tb = *Mycobacterium tuberculosis*.

^c Four elephants (herd F) pending culture.

^d Twenty-one samples had multiple isolates.

French rubber catheter (Sovereign, Sherwood Medical, St. Louis Missouri 63103, USA) into one nostril, instilling 30–60 ml of sterile saline, and then aspirating into a syringe. The second method consisted of instilling 60 ml of saline through a catheter tip syringe (Monoject, Sherwood Medical) into one nostril and collecting the exhaled material into a clean 3.78-L plastic bag held over the end of the trunk. The latter method yielded significantly more

material and was the method of choice for subsequent samplings for all herds. Drinking water was typically withheld for 2 hr prior to sampling. Subsequent to the distribution of published guidelines,³⁵ three trunk wash samples, collected on separate days within a 1-wk period, were submitted for culture. Oropharyngeal and trunk swabs were collected on standard culture swabs (Culturette, Becton Dickinson, Cockeysville, Maryland 21030, USA).

Table 2. History of elephants culture positive for *Mycobacterium tuberculosis* as of December 1999.

Elephant	Age (yr)	Date of diagnosis	Clinical signs related to tuberculosis	Pretreatment or antemortem hematology and serum chemistry findings ^{a,b}	Treatment status
A1	47	Aug 1996	weight loss; appetite loss	↓ PCV	NA ^c (postmortem diagnosis)
A2	26	Aug 1996	none	WNL	NA (postmortem diagnosis)
A4	46	Dec 1996	weight loss	WNL	completed Dec 1997
B1	30	Mar 1997	none	WNL	NA (postmortem diagnosis)
C1	29	May 1997	none	WNL	completed Dec 1998
D1	32	May 1997	none	↑ lymphocytes	completed Jun 1998; second treatment course ongoing
D2	30	May 1997	nasal discharge	↑ lymphocytes	completed Jun 1998
E1	10	Aug 1998	none	↑ WBC ↑ PCV ↑ TP	completed Oct 1999
FB1	32	May 1998	none	WNL	ongoing
FB2	32	Nov 1998	none	WNL	ongoing
FR3	45	Sep 1998	weight loss	WNL	NA (postmortem diagnosis)
FR4	52	Sep 1999	weight loss	WNL	NA (euthanatized Oct 1999)

^a Values compared with ISIS normals (ISIS, 12101 Johnny Cake Ridge Road, Apple Valley, Minnesota 55124, USA).

^b PCV = packed cell volume; WNL = within normal limits; WBC = white blood cell count; TP = total protein.

^c NA = not applicable.

Fecal samples were collected from freshly voided stools.

Nucleic acid amplification test (NAAT)

Sputum obtained from trunk washes or swabs was submitted for nucleic acid amplification to the NVSL (herds A, E, and F); the Massachusetts State Laboratory, Tuberculosis Division, (Boston, Massachusetts 02130, USA; herd A); and the County of Orange Health Care Agency, Public Health Laboratory (herds B, C, D, and F). All laboratories employed the Gen-Probe Amplified *M. tuberculosis* Direct Test (MTD; Gen-Probe, San Diego, California 92121, USA), which utilizes transcription mediated amplification to specifically replicate ribosomal RNA from bacteria of the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, and *M. microti*).

Intradermal tuberculin testing

The i.d. tuberculin test was administered to elephants in herds A, C, D, and E. All tuberculin testing was conducted prior to treatment with anti-tuberculosis drugs. In October 1996, herd A elephants were tested in the caudal ear fold with 0.1 ml purified protein derivative (PPD) prepared from *M. bovis* (PPD bovis, 1 mg protein/ml, Coopers Animal Health, Inc., Kansas City, Kansas 66103-1438, USA). In December 1996, 12 herd A elephants were tested with 0.1 ml PPD bovis prepared for use in the cervical skin test (Cervical Test SR 31-CER 9601, USDA); however, the PPD was injected into the caudal ear fold. Herd C was tested with 0.1 ml PPD bovis tuberculin in the ear fold in April 1997. Saline (0.1 ml) was injected into the opposite ear as a control.

Herd D was tested with 0.1 ml PPD bovis tuberculin in the tail fold in August 1996 and October 1996 and with 0.2 ml PPD bovis tuberculin in the caudal ear fold in May 1997. Sterile saline (0.2 ml) was injected into the opposite ear as a control.

Intradermal tuberculin test results for herds A, C, and D were determined by palpation at 72 hr.

Herd E was tested with 0.1 ml of PPD prepared from *M. avium* (avian PPD, 0.4 mg protein/ml) balanced tuberculin (USDA), 0.1 ml bovine PPD balanced tuberculin (USDA), and 0.1 ml of saline in the caudal ear fold in October 1998. Pre- and post-injection skin thickness was measured, and results were plotted on a comparative cervical tuberculin test scattergram used for domestic cattle and Cervidae.³⁰

ELISA

Serum collected pretreatment and prior to i.d. tuberculin testing was submitted to three independent

research laboratories for ELISA testing: lab 1 (College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523, USA), lab 2 (Department of Microbiology, Colorado State University), and lab 3 (Iowa State University, Ames, Iowa 50011, USA). Staphylococcal protein A (SPA) was used to detect the presence of bound antibody on ELISA plates in the three labs.

Lab 1 used a six-antigen panel comprised of culture filtrate (CF; a sterile, heat-killed filtrate of *M. bovis*, strain AN5), bovine BPPD, MBP70, an *M. bovis*-specific antigen derived from strain AN5, two lipoarabinomannan (LAM) antigens derived from virulent strains of *M. tuberculosis* (Erdman and H37Ra [RA]), and avian PPD derived from *M. avium*.¹² The ELISA was performed by techniques previously described.²⁰ Elephants were classified as positive or negative on the basis of seroreactivity (as quantified by optical density) to CF and RA.²⁰

Lab 2 used a three-antigen panel consisting of culture filtrate protein, LAM, and *M. avium*. Elephants were classified as positive or negative on the basis of optical density (O.D.) measurements of LAM and CF at 490 nm. An optical density ≤ 0.2 was considered negative, an O.D. of 0.2–0.5 was designated weak positive (suspect), and an O.D. ≥ 0.5 was interpreted as strong positive.

Lab 3 used a modification of a previously reported ELISA assay.³⁴ The four-antigen panel was comprised of N-lauryl-sarcosyl extract of *M. bovis*, *M. bovis* PPD, *M. avium* PPD, and *Mycobacterium intracellulare* PPD. Interpretation was based on reactions in sera diluted 1:10, 1:20, 1:40, and 1:80 by comparison with known positive and negative reference sera from the Iowa State University sera bank. Reaction to homologous antigens of *M. bovis* that were two times greater than reactions observed to either the *M. avium* or *M. intracellulare* antigens were considered positive.

ELISA testing performed as part of the blood tuberculosis (BTB) test is discussed below.

BTB test

Whole blood from five herds (A–E) was submitted to the Department of Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843-4467, USA. The BTB test is a composite test consisting of ELISA reactivity against mycobacterial antigens and lymphocyte stimulation. Results are determined as a weighted average of these measurements.¹⁶ Cells that are reactive to a positive control by lymphocyte stimulation may be reported as bovine, avian, negative (no dominant reaction), or equivocal (equal

Table 3. Reported isolations other than *Mycobacterium tuberculosis* from six elephant herds.

Mycobacterial species	Number of isolations					
	Herd A	Herd B	Herd C	Herd D	Herd E	Herd F
<i>Mycobacterium abscessus</i>	—	—	1	—	—	1
<i>Mycobacterium aurum</i>	—	—	—	1	—	—
<i>Mycobacterium avium</i>	2	4	1	24	34	96
<i>Mycobacterium chitae</i>	—	—	1	—	—	—
<i>Mycobacterium chelonae</i>	—	—	—	—	1	1
<i>Mycobacterium flavescens</i>	—	1	—	2	—	3
<i>Mycobacterium fortuitum</i>	—	1	9	10	—	19
<i>Mycobacterium gastri</i>	1	—	—	—	—	1
<i>Mycobacterium gordonae</i>	—	—	—	1	1	8
<i>Mycobacterium intracellulare</i>	—	—	1	—	—	—
<i>Mycobacterium lentiflarum</i>	—	—	—	—	—	1
<i>Mycobacterium mucogenicum</i>	—	—	—	—	—	3
<i>Mycobacterium neoaurum</i>	—	1	—	—	—	—
<i>Mycobacterium phlei</i>	—	—	—	—	—	1
<i>Mycobacterium scrofulaceum</i>	—	—	—	—	—	2
<i>Mycobacterium simiae</i>	—	—	—	—	—	1
<i>Mycobacterium smegmatis</i>	—	1	—	1	—	—
<i>Mycobacterium terrae</i>	—	—	—	—	3	5
<i>Mycobacterium ulcerans</i>	—	—	—	1	—	2
<i>Mycobacterium xenopi</i>	—	—	—	1	—	1
Group IV <i>Mycobacterium</i>	1	—	—	—	—	2
Unspecified species	—	6	5	41	73	45
<i>Nocardia</i>	—	—	—	12	5	4
Total	4	14	18	94	117	195

reactions). Samples with nonreactive cells are reported as “no data.”

Lymphocyte stimulation was assessed by measurement of the uptake of ³H-thymidine after the addition of mycobacterial antigens into a cell culture of each elephant's peripheral blood mononuclear cells. Purified protein derivatives from bovine and avian strains were used as antigens. Control wells containing concanavalin-A, as a nonspecific mitogen, were included as a positive control. The ELISA reactivity was measured against bovine and avian PPDs. Prior to June 1997, antibody detection for ELISA testing was performed with anti-bovine antibody. After June 1997, SPA was used to detect bound elephant antibodies.

Molecular evaluation

Restriction fragment length polymorphism (RFLP) was conducted at the USDA, Agricultural Research Service, National Animal Disease Center, Ames, Iowa 50010, USA. *Mycobacterium tuberculosis* isolates from 12 elephants were received from the USDA, APHIS, NVSL, Ames, Iowa 50011, USA. Isolates were subcultured, DNA was extracted, and Southern hybridizations were con-

ducted by previously published methods.^{41,44} The DNA was digested with restriction endonuclease *Pvu*II site.⁹ This method is generally accepted as the standard for RFLP analysis of *M. tuberculosis* isolates.⁴⁰

Statistical analyses

Sensitivity and specificity calculations for diagnostic tests were calculated by standard methods²² with data from culture results as the “gold standard.” To allow for direct comparisons between ELISA methods, “weak positive” results were excluded from sensitivity and specificity calculations. Associated 95% confidence intervals were also calculated for these values.^{10,18} The degree of agreement between trunk cultures and nucleic acid amplification tests was assessed through calculation of the Kappa statistic (and associated statistical significance).⁶ Data analysis was accomplished with Epi Info 6.04b (Centers for Disease Control, Atlanta, Georgia 30333, USA).

RESULTS

Results are reported in Tables 1–7. True positives were defined as elephants from which *M. tubercu-*

Table 4. Comparison of nucleic acid amplification and trunk culture results in six herds of elephants.^a

Herd	No. M.tb culture positive	NAAT			No. M.tb culture negative	NAAT	
		+	-	NE		+	-
A	NE	NE	NE	NE	28	0	28
B	NE	NE	NE	NE	37	0	37 ^b
C	4	4	0	8	8	0	8
D	NE	NE	NE	NE	73	2 ^c	71
E	2	1	1	10	10	0	10
F	9	9	0	445	18 ^d		427 ^e
Total	15	14	1	601	20		581

^a M.tb = *Mycobacterium tuberculosis*; NAAT = nucleic acid amplification test; NE = none examined.

^b Two samples overgrown.

^c *Nocardia* and *Mycobacterium fortuitum* isolated from one sample.

^d Atypical or unidentified mycobacteria isolated from eight samples; seven samples overgrown.

^e Twenty-eight samples overgrown.

losis was isolated (i.e., culture was used as the “gold standard” diagnostic method). True negatives were defined as elephants from which *M. tuberculosis* was not isolated. The calculated values are based on the assumption that culture will accurately discriminate between infected and noninfected animals. A number of variables may limit the validity of this assumption. Culture overgrowth by contaminating organisms, inadequate sample volume, or errors in sample handling may result in a false-negative culture. Culture may also fail to detect infected animals that are not shedding at the time of sampling. Culture has been reported to have a specificity of 100% in humans but a lower sensitivity, which may be of greater concern.⁴³

Culture

Mycobacterial culture results of the six herds are reported in Table 1. *Mycobacterium tuberculosis* was isolated from 12 elephants, four of which were dead (A1, A2, B1, and FR3). A total of 2,105 in-

dividual samples yielded 33 *M. tuberculosis* cultures. One hundred thirty-five samples (6.8%) were overgrown.

Of 153 herd E samples processed by the Arkansas Department of Health, 47.7% were overgrown on conventional media, whereas only 1.97% were overgrown on BACTEC culture (Becton-Dickinson, Sparks, Maryland 21152, USA). Although most mycobacterial laboratories utilize both conventional and BACTEC media, results are not generally reported separately. Further, some laboratories do not indicate if cultures are overgrown (i.e., overgrown cultures are reported as “no isolation”).

The histories of the 12 *M. tuberculosis*-infected elephants are summarized in Table 2. Ten of the positive elephants, including the four that were diagnosed postmortem, were female Asian elephants with a mean age of 37 yr (26–52 yr); two were male Asian elephants (10 and 32 yr).

Mycobacterium tuberculosis was cultured from three elephants after treatment was started. Screen-

Table 5. Results of the tuberculin test, blood tuberculosis (BTB) test, and enzyme-linked immunosorbent assay (ELISA) for *Mycobacterium tuberculosis* culture-positive elephants in six herds.^a

Herd	Tuberculin test			BTB test			ELISA ^b								
							Lab 1			Lab 2			Lab 3		
	n	S	NR	n	+	-	n	+	-	n	+	-	n	+	-
A	1	0	1	1	1	0	1	1	0	1	0	1	1	0	1
B	NE	NE	NE	1	0	1	1	1	0	1	1	0	1	1	0
C	2	0	2	1	1	0	1	1	0	NE	NE	NE	1	1	0
D	2	0	2	2	2	0	2	2	0	NE	NE	NE	2	2	0
E	1	1	0	1	1	0	1	1	0	1	0	1	NE	NE	NE
F	NE	NE	NE	NE	NE	NE	2	2	0	NE	NE	NE	3	3	0
Total	6	1	5	6	5	1	8	8	0	3	1	2	8	7	1

^a n = number of elephants tested; S = suspect; NR = nonresponder; NE = not examined.

^b Blood samples for ELISA were collected prior to intradermal tuberculin testing.

Table 6. Results of the tuberculin test, blood tuberculosis (BTB) test, and enzyme-linked immunosorbent assay (ELISA) for *Mycobacterium tuberculosis* culture-negative elephants in six herds.^a

Herd	Tuberculin test			BTB test			ELISA ^b											
							Lab 1				Lab 2				Lab 3			
	n	S	N	n	+	-	n	+	-	±	n	+	-	±	n	+	-	±
A	17	6	11	11	9	2	17	1	16	0	17	0	13	4	17	1	12	4
B	NE	NE	NE	3	0	3	4	1	3	0	4	0	3	1	4	0	4	0
C	1	0	1	2	0	2	1	0	1	0	NE	NE	NE	NE	1	0	1	NE
D	4	0	4	7	3	4	2	1	1	0	NE	NE	NE	NE	2	0	1	1
E	9	2	7	8	3	5	9	0	9	0	9	0	9	0	NE	NE	NE	NE
F	NE	NE	NE	NE	NE	NE	8	2	6	0	NE	NE	NE	NE	50	8 ^c	28	14
Total	31	8	23	31	15	16	41	5	36	0	30	0	25	5	74	9	46	19

^a n = number of elephants tested; S = positive test result; N = negative test result; NE = not examined.

^b ELISA samples collected prior to intradermal tuberculin testing.

^c Weak positive reaction.

ing and pretreatment cultures of elephant C1 obtained 36 and 11 days before starting therapy as well as six of eight cultures obtained 29–38 days after the initiation of treatment were positive. The subsequent 36 cultures from day 53 of treatment were negative. Elephant FB2 had a positive culture 3 days after the initiation of treatment but no isolation from a sample taken 25 days later or from subsequent samples. Elephant FR4 had a positive culture 18 days after starting therapy.

Mycobacterium tuberculosis was isolated from elephant A3 40 and 4 days pretreatment. The first follow-up trunk washes on treatment day 50 were negative as were all subsequent cultures. Elephants D1, D2, and E1 had negative cultures prior to the initiation of treatment. Elephant D1 was culture positive 15 and 7 days prior to treatment but was culture negative the day treatment was initiated, the subsequent 2 days, and throughout the course of treatment. For animal D2, a single culture 15 days pretreatment was positive; however, trunk washes on treatment days 1, 2, and 3 and throughout treatment were negative. Elephant E1 did not start treat-

ment until 120 days after the positive screening culture and had a negative culture at the time therapy was initiated. All subsequent cultures were negative.

Isolations other than *M. tuberculosis* are listed in Table 3. Four hundred forty-two samples from 81 elephants (68.6%) yielded either *Nocardia*, mycobacteria other than *M. tuberculosis* or unidentified mycobacteria. Seven elephants that were culture positive for *M. tuberculosis* had additional mycobacteria isolated. *Mycobacterium avium* was the most common isolate.

Drug susceptibility tests

Drug susceptibility tests were performed on *M. tuberculosis* isolates from elephants A1, C1, D1, FB1, FB2, and FR4. All strains were sensitive to isoniazid (INH), rifampin (RIF), ethambutol, streptomycin, and pyrazinamide (PZA) with the exception of the isolate from elephant FR4 that was INH resistant. Elephant FR4 had been treated for 12 mo during 1994–1995 with rectally administered INH on the basis of clinical observations of weight loss.

Table 7. Sensitivity and specificity of the intradermal tuberculin test, three enzyme-linked immunosorbent assays (ELISAs), and the blood tuberculosis (BTB) test to predict *Mycobacterium tuberculosis* in six elephant herds (with culture as the “gold standard” diagnostic method).

Test	Total analyses	Sensitivity (95% CI)	Specificity (95% CI)
Tuberculin test	37	16.7% (0.9–63.5%)	74.2% (55.1–87.5%)
Lab 1 ELISA ^a	49	100% (48.2–100%)	87.8% (67.8–96.5%)
Lab 2 ELISA ^a	28	33.3% (1.1–91.5%)	100% (76.0–100%)
Lab 3 ELISA ^a	63	87.5% (37.2–99.6%)	83.6% (66.3–93.3%)
BTB	37	83.3% (36.5–99.1%)	51.6% (33.4–69.4%)

^a Exclusive of “weak positives.”

Nucleic acid amplification

A total of 616 samples had both culture and amplification results (Table 4). Fourteen culture-positive samples were MTD positive and one culture-positive sample was MTD negative. Twenty culture-negative samples were MTD positive. Atypical or unidentified mycobacteria were isolated from nine of these. One culture-negative, MTD-positive specimen was from animal D1 on day 2 of treatment and may reflect the detection of dead organisms or a low inoculum (<100 organisms/ml).

There were 581 *M. tuberculosis* culture-negative samples that were also MTD negative. Because multiple samples were collected from individual animals, assessment of the sensitivity and specificity for the NAAT could not be calculated. Comparison of results between culture and NAAT, however, showed that there was a statistically significant 96.6% agreement between the two methods (Kappa = 0.556, standard error = 0.037; $P < 0.000001$).

Intradermal tuberculin testing

Tuberculin test results are reported in Tables 5 and 6. Tuberculin testing was performed in herds A, C (Asians only), D, and E. Of six *M. tuberculosis*-positive elephants, one (E1) had a suspect tuberculin reaction. This elephant had been tested with balanced tuberculin antigens. Of the 31 *M. tuberculosis* culture-negative elephants tested, eight (26%) showed suspect reactions and 23 were non-responders. The sensitivity and specificity of the tuberculin test were 16.7% (95% confidence interval [CI] = 0.9–63.5%) and 74.2% (95% CI = 55.1–87.5%), respectively (Table 7).

There was no apparent difference in test reactivity between Asian elephants and African elephants. Two of eight (25%) African elephants had suspect reactions, whereas 8 of 27 Asian elephants (30%) were suspect.

Pretreatment tuberculin testing was performed repeatedly for 14 elephants: twice for 12 elephants of herd A and three times for four elephants of herd D. Two elephants gave consistent suspect reactions, eight elephants were consistent nonreactors, and two elephants were initially nonreactive and then had suspect reactions.

ELISA

Pretreatment ELISA testing was performed at three laboratories. ELISA results from eight *M. tuberculosis* culture-positive elephants and 74 culture-negative elephants were available, although not all laboratories analyzed all available samples

(Tables 5, 6). Sensitivities of the three ELISA methods were 33.3–100% (95% CI: 1.1–100%). Specificities were 83.6–100% (95% CI: 66.3–100%).

BTB test

The BTB test was performed on five herds (Tables 5, 6). The BTB test was interpreted as follows to determine sensitivity and specificity: reactivity to bovine antigen was considered positive (on the basis of known cross-reaction between *M. tuberculosis* and *M. bovis*); a negative result or reactivity to avian PPD was considered negative; and equivocal and no data results were excluded. For six culture-positive animals, initial results from five animals showed *M. bovis* reactivity, giving a sensitivity estimate of 83.3% (95% CI: 36.5–99.1%). Of the 31 culture-negative animals tested, 15 returned positive BTB results, giving a specificity for the BTB test of 51.6% (95% CI: 33.4–69.4%; Table 7).

Repeat pretreatment testing was performed on herd A and elephants B2, B3, and C1. For the two culture-positive elephants (A1 and C1), results from only one (C1) were consistently reported as bovine, the other as bovine and no data. For the 17 culture-negative elephants in this group, only five gave consistent results. Four elephants were consistently reported as bovine and one as equivocal. Of the 12 test results that differed, four were reported as avian and bovine on different test dates, one elephant had equivocal, negative, and nonspecific results on three pretreatment tests, and one had avian and equivocal results on two pretreatment tests. Two herd B elephants (B4 and B5) that were not treated also had inconsistent BTB test results.

Molecular epidemiology

Results of RFLP analysis are shown in Figure 1. Isolates from seven living elephants (A3, C1, D1, D2, E1, FB1, and FB2) and five deceased elephants (A1, A2, B1, FR3, and the herd A elephant that died in 1994) were available for analysis. The RFLP pattern for the *M. tuberculosis* isolate from the herd A elephant that died in 1994 was identical to the patterns for elephants A1 and A3 (Fig. 1, lanes 2–4). There was a one-band difference between the isolate from A2 and the other isolates from herd A elephants (Fig. 1, lane 5). Thus, all isolates from herd A elephants were considered to represent highly related strains.

The RFLP patterns for isolates from elephants B1 and C1 were identical to each other and differed by one band from isolates from elephants D1 and D2 (Fig. 1, lanes 6, 7, 10, 11). The isolate from elephant E1 had an RFLP pattern that differed by two bands when compared with isolates from ele-

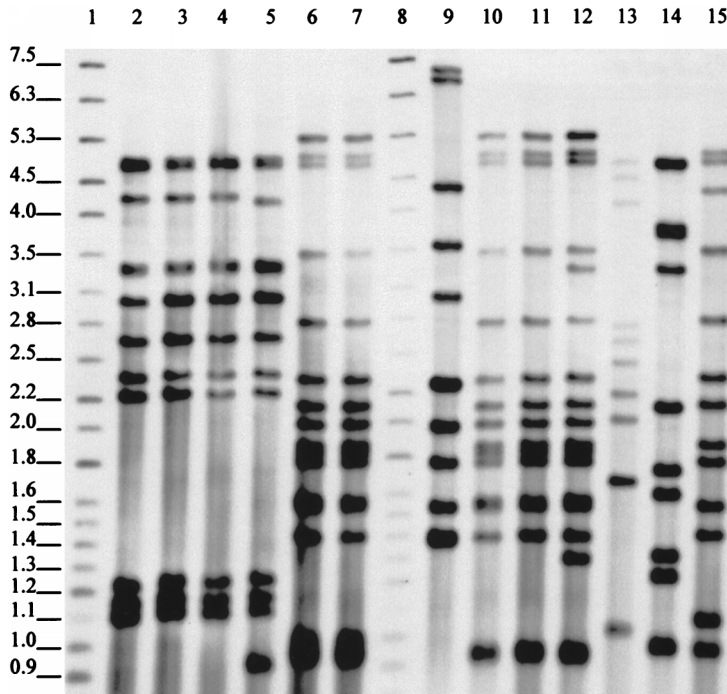


Figure 1. RFLP analysis of *Mycobacterium tuberculosis* isolates from elephants. DNA was digested with restriction endonuclease *Pvu*II and hybridized with IS6110. The identities of elephants from which *M. tuberculosis* was isolated are as follow: Lanes 1 and 8, molecular size markers (provided by J. T. Crawford); lane 2, herd A elephant that died in 1994; lane 3, A1; lane 4, A4; lane 5, A2; lane 6, D2; lane 7, D1; lane 9, *M. tuberculosis* reference strain 14323; lane 10, B1; lane 11, C1; lane 12, E1; lane 13, F1; lane 14, F2; and lane 15, F3.

phants B1 and C1 (Fig. 1, lanes 10, 12). When DNA from these *M. tuberculosis* isolates was digested with *Alu*I and probed with a polymorphic G-C rich repetitive sequence (PGRS), which is considered a secondary method for strain typing,⁴² the patterns for isolates from elephants B1, C1, D1, D2, and E1 were identical (data not shown). These results indicate that the *M. tuberculosis* isolates from these elephants are closely related.

The isolate from elephant FR3 was considered distinct. Although the RFLP pattern for the FR3 isolate had 11 bands that matched the isolates from elephants B1, C1, D1, D2, and E1, several bands did not match. The PGRS pattern was also similar to the pattern for the other isolates; however, some of the bands did not match the other patterns. These results indicate that there may be some relationship between the isolates from elephant FR3 and the isolates from elephants B1, C1, D1, D2, and E1, but that relationship may be distant.

The RFLP patterns for *M. tuberculosis* isolates from elephants FB1 and FB2 did not match each other, nor did they match any of the patterns from the other elephant isolates.

THERAPEUTIC APPROACHES

Each of the herds had elephants that underwent treatment for *M. tuberculosis* infection. In general, treatment consisted of combinations of isoniazid (INH; herds A, B, C, D, E, and F), rifampin (RIF; herds A, B, C, and D) and pyrazinamide (PZA; herds A, C, E, and F). Initial daily drug dosages were extrapolated from human protocols, which recommend INH at 5 mg/kg, PZA at 25 mg/kg, and RIF at 8 mg/kg.¹ Vitamin B6 (pyridoxine hydrochloride) was administered at a dosage of 1–1.5 mg/kg/day (herds A, B, C, and D) to prevent INH-related peripheral neuropathies.¹⁴ Drugs given orally were formulated by Congaree Veterinary Pharmacy (West Columbia, South Carolina 29170, USA).

Herd A

One culture-positive elephant and 18 exposed herd mates were treated. Treatment of herd A was initiated in December 1996. Fourteen elephants were placed in a “high-risk” treatment group and four in a “low-risk” treatment group on the basis

of the degree of exposure to known culture-positive elephants. The high-risk group received INH, PZA, and RIF daily for 2 mo and INH and RIF four times weekly for 10 mo. The low-risk group received INH and RIF daily for 2 mo then four times weekly for 4 mo. Drugs were given over food to adult elephants and in a bottle with milk to a 3-yr-old hand-raised male. In August 1997, treatment of the culture-negative animals was stopped on the basis of recommendations of the Elephant Advisory Panel that culture-negative, exposed animals receive 6 mo of drug therapy. Drug doses were increased to 7.5 mg/kg (INH), 30 mg/kg (PZA), and 10 mg/kg (RIF), respectively, on the basis of results of pharmacokinetic trials (to be reported separately). Treatment of culture-negative elephants was completed in August 1997. Treatment of culture-positive elephant A3 was completed in November 1997. No adverse reactions to treatment were noted. Acceptance of medications over food was variable and drug levels were often low.

Herd B

Exposed Asian elephants B2 and B3 and African elephants B4 and B5 were treated for 6 mo beginning July 1997. Isoniazid (7.5 mg/kg), RIF (8 mg/kg), and PZA (25 mg/kg) were given once daily for 60 days then four times weekly. Medications were mixed with food and given orally. Acceptance of medications over food was variable and drug levels were low. No adverse reactions to the treatment were noted.

Herd C

One culture positive elephant (C1) and one exposed elephant (C2) were treated. Two African elephants (C3 and C4) were considered nonexposed and were not treated. Treatment of elephant C1 was initiated in May 1997 with INH (7.5 mg/kg), RIF (8 mg/kg), and PZA (25 mg/kg) given over food. After 6 wk, oral treatment was discontinued because of poor acceptance. Treatment was completed via rectal suppository (formulated by Abbott's Medical Pharmacy, Berkeley, California 94705, USA) with INH and RIF for 6 mo, then INH and PZA four times a week for 6 mo. On the basis of pharmacokinetic results, the dose of INH was decreased from 7.5 mg/kg to 2.5 mg/kg and PZA was increased from 25 mg/kg to 35 mg/kg. Rifampin was discontinued after the first 6 mo of treatment because of the persistent failure to reach minimal proposed therapeutic levels at a dose range of 8–16 mg/kg.

Elephant C1 completed treatment December 1998. This elephant exhibited partial anorexia with

orally administered INH, RIF, PZA, and B6, which made it difficult to administer medications consistently. Rectally administered INH at a dose of 11.5 mg/kg produced partial anorexia, lethargy, and pica (eating dirt). Yellow brown urine was also observed. Liver toxicity was evidenced by elevations in serum AST, total bilirubin, GGT, and bile acids. After the discontinuation of treatment, appetite, behavior, and activity level returned to normal within 2–3 days, and liver enzymes returned to normal after 4 wk. Daily treatment with INH was then re-instituted with a dose of 3.75 mg/kg. Lethargy and loss of appetite recurred if the dose was increased to 5 mg/kg or greater. When INH and PZA were administered four times weekly, a low-grade anemia was observed. The anemia resolved when the INH dose was decreased from 3.75 to 2.5 mg/kg and the PZA dose was decreased from 35 to 25 mg/kg.

Elephant C2 was treated prophylactically with INH (7.5 mg/kg) administered orally over food. After 5 mo, treatment was discontinued because of poor acceptance and failure to reach minimal proposed therapeutic levels.

Herd D

Two culture-positive (D1 and D2) and two exposed elephants (D3 and D4) were treated. Elephants D5–D7 were considered low risk and were not treated. Treatment with INH (7.5 mg/kg) and RIF (9.9 mg/kg) was initiated in June 1997. All medications were delivered orally by direct dose syringe except for a brief period when D4 was treated by rectal suppository.

Elephants D1, D2, D3, and D4 developed inappetence, lethargy, and pica. On the basis of high therapeutic blood levels and the obvious physical discomfort of all four elephants, dosage levels were adjusted to 5.6 mg/kg (INH) and 7.5 mg/kg (RIF) in July 1997. All four elephants were treated daily for the first 3 mo, then four times per week. In September, elephant D4's total WBC count decreased from 13,000/ μ l to 1,900/ μ l at which time INH was discontinued for both elephants D3 and D4. Rifampin was continued at 6 mg/kg through December 1997, when treatment was completed. Elephants D1 and D2 continued INH at 5.6 mg/kg and RIF at 7.5 mg/kg four times per week through March 1998. Between April and June 1998, elephants D1 and D2 received INH at 7.5 mg/kg and RIF at 9.9 mg/kg, on the basis of blood levels, at which time treatment was considered completed.

All cultures were negative until December 1998 when elephant D1 (who had completed treatment in June 1998) produced a solitary mucous plug that

yielded acid-fast organisms on smear and *M. fortuitum* and *M. tuberculosis* on culture. Subsequent cultures of this animal and her herd mates were negative.

Herd E

One culture-positive elephant (E1) was treated. Culture-negative herd E elephants were cultured every other month according to the USDA Guidelines for Group C animals (negative culture, exposure to culture-positive animal within last 12 mo). Treatment of elephant E1 commenced in October 1998 with INH (10 mg/kg/day). Within 3 wk of the initiation of treatment with INH alone, elephant E1 showed lethargy, inappetance, and an elevation in LDH. Isoniazid was discontinued for 1 mo, then reinstated at 5 mg/kg. Pyrazinamide was added in January 1999 (25 mg/kg/day). Pyrazinamide was given daily for 3 mo, then every other day for 4 mo. Both drugs were administered rectally. Elephant E1 experienced several episodes of lethargy and inappetance that resolved after treatment was discontinued for 1–2 wk. Reinitiating INH at a half-dose and increasing to a full dose over a period of several weeks was effective in preventing further problems. Isoniazid was given four times weekly beginning September 1999. Treatment of elephant E1 was completed in October 1999.

Herd F

Two culture-positive elephants (FB1 and FB2) are under treatment with rectally administered INH and PZA. Treatment of FB1 was initiated July 1998. Elephant FB2 began treatment in May 1999. Dosages varied on the basis of drug levels achieved in individual elephants. Nine FB elephants that were culture negative but MTD positive were treated prophylactically with INH or INH and RIF. Elephant FR4 was euthanatized in October 1999.

DISCUSSION

In this study, we examined a total of 118 elephants (105 Asian elephants and 13 African elephants). Twelve elephants were culture positive for *M. tuberculosis*. Although all culture-positive individuals were Asian elephants, the small number of African elephants in the study precludes any conclusions concerning species susceptibility.

Among humans, exposure to an individual who is actively shedding *M. tuberculosis* may result in five possible scenarios: no infection (the exposure was not relevant), infection and development of acute disease, infection and eradication of infection from the body, infection with containment of the infection and the development of latent (quiescent)

infection, or development of latent infection that reactivates at some later date. For humans, the last is the most likely cause of active disease. The latter four scenarios will result in reactivity to mycobacterial antigens on i.d. testing (though late-stage anergy is possible).

Intradermal tuberculin testing with PPD antigen is recommended for humans exposed to TB. If PPD positive, a chest radiograph is performed, and if suggestive of TB, sputum is obtained for culture. If the person is PPD positive but radiographically and/or culture negative, then the disease is considered latent. Active disease is associated with a positive culture. In greater than 95% of cases, treatment is associated with cure assuming a susceptible organism and compliance with the treatment regimen.³

The pathophysiology of TB in elephants has not been well studied. Most cases in the literature involve advanced pathologic changes. This may be due to the lack of early antemortem diagnosis. Prior to the USDA requirement, fewer than 50% of the elephants in the U.S. were routinely tested for TB, and the i.d. tuberculin test was the method typically used.²⁵

The Elephant Tuberculosis Advisory Panel faced the challenge of establishing diagnostic and effective treatment regimens for elephants, a situation that was without precedent. By empiric trial, trunk washes appeared to yield the best material for culture that could be obtained without anesthesia. Instillation of sterile saline into the trunk of conditioned elephants and collection into a plastic bag yielded better recovery of sample than reaspiration of trunk contents or swab. Because the TB organism can be shed intermittently, a series of three samples within a 1-wk period was recommended. Proper sample handling and immediate shipment (or freezing the sample if shipment is delayed) will minimize overgrowth. Mycobacterial culture requires 8 wk, and although considered the method of reference, accuracy may be compromised by improper sample handling, contamination, or laboratory error.

As an adjunct to culture, the NAAT (or MTD) test appeared to yield results comparable to those reported for humans.^{26,42,43} Additionally, culture and MTD showed excellent statistical agreement in this study. Twenty samples were culture negative for *M. tuberculosis* and MTD positive. Nontuberculous mycobacteria were isolated from nine of these samples. Although false-positive MTD results in humans have been attributed to low O.D. values, four of seven clinically negative human specimens in one study yielded *M. kansasii*.⁷ Because amplification methods are currently FDA approved only

for smear positive (acid-fast) samples in humans, it seems reasonable to consider this test as useful but experimental in elephants. As with humans, amplification techniques would seem to be able to rule in, but not rule out, infection in specimens with small numbers of organisms (i.e., smear negative).

Of six *M. tuberculosis* culture-positive elephants, only one showed reactivity to i.d. tuberculin, whereas 26% of culture-negative elephants showed reactivity. Thus, despite the agreement between test results for animals that had repeated tests performed and the apparent lack of PPD conversion by exposure to prior tuberculin test antigens, there was little correlation between skin test results and active infection.

The BTB test demonstrated a sensitivity of 83.3% and a specificity of 51.6%. However, there was poor consistency for elephants that had repeat testing prior to the initiation of treatment. This test is no longer available in the United States.

The ELISA testing performed before i.d. tuberculin injection showed good sensitivity and specificity (Table 7), with all laboratories showing statistically similar results. Lab 2 appears to have a much lower sensitivity than Lab 1 or Lab 3; however, Lab 2 performed ELISA testing on only three culture-positive elephants, which is evidenced by the extremely large confidence interval associated with the estimate. All three labs had similar specificities; the variability between laboratories may have been due to the use of different antigens, different techniques, or differences in sample size. There were also differences in "cut-off" values because Lab 2 and Lab 3 had culture-negative animals that were designated as weakly positive (\pm), whereas Lab 1 did not include this category. Additional investigations with these three methods are warranted before any conclusions regarding their validity are made.

The practical application of ELISA in the diagnosis of tuberculosis in elephants remains to be determined. Several elephants that were trunk culture negative had positive ELISA results (Table 6). The reasons for this discrepancy are unknown but may be due to undetected infection or nonspecific reactions. Nonspecific reactions may wane on repeated ELISA testing and may be associated with exposure to *M. avium*, *M. intracellulare*, or saprophytic environmental mycobacteria.

It may be desirable to have paired serum samples collected at 4–6-mo intervals to determine the significance of ELISA reactions in animals from collections that have no history of tuberculosis infection. Furthermore, in order to improve overall test validity, it may be useful to evaluate ELISA results

in animals before and after i.d. injection of tuberculin. In cervids, the sensitivity of the ELISA can be dramatically improved if it is evaluated 10 days after an i.d. tuberculin test.¹⁵ However, a study in four culture-negative elephants showed increased reactivity of ELISA after i.d. testing.²⁷ Until further data are collected and evaluated, ELISA results should be evaluated in context with other test results.

The elephant from herd A that died in 1994 was infected with the same strain of *M. tuberculosis* that infected elephants A1 and A3. An elephant handler that worked with herd A was also infected with the same strain.²⁴ Elephant A2 was infected with a closely related strain of *M. tuberculosis*. Although the elephants and handler were infected with a common strain of *M. tuberculosis*, it is not possible to determine the source of infection. The DNA fingerprints of the *M. tuberculosis* isolates from herd A were not found in any of the other elephant isolates.

Elephants B1, C1, D1, and D2 were infected with an identical strain of *M. tuberculosis*, and elephant E1 was infected with a closely related strain. Although the source of infection in these elephants cannot be determined, these elephants were all housed together at facility D from September 1993 through February 1994, suggesting a possible epidemiologic link. The *M. tuberculosis* infection in elephant FR3 may also be linked to these cases although the relationship is more distant than the others. Although elephants FB1, FB2, and FR3 were all from the same herd, they were infected with different strains of *M. tuberculosis*, indicating different sources of infection.

The one study that measured INH levels in an elephant⁸ provided a starting point for a therapeutic plan based on current human treatment regimens. Five of seven living culture-positive elephants have completed treatment. Of these, one (D1) is undergoing a second course of treatment subsequent to an *M. tuberculosis*-positive culture 5 mo after completing the first treatment course. Further monitoring over time will be required to validate treatment protocols for elephants.

The true prevalence of TB in elephants is unknown. Between August 1996 and October 2000, *M. tuberculosis* was isolated from 18 elephants in the United States. On the basis of 539 elephants in the NVSL database (Dr. Janet Payeur, pers. comm; note that 532 elephants are identified by the North American Regional Studbook Keepers, Deborah J. Olson, Mike Keele, pers. comm.), these 18 cases represent an estimated prevalence of 3.3%.

It is imperative that antemortem tests for tubercu-

losis are performed before any elephant is euthanized. All elephants that die should be thoroughly necropsied and examined for tuberculous lesions regardless of premortem culture status. Consultation with experienced prosectors is recommended to ensure that lymph nodes are properly identified and sampled for histopathology and culture. The Elephant Necropsy Protocol is available, together with Guidelines for the Control of Tuberculosis in Elephants, at <http://www.aphis.usda.gov/ac/acindex.html>.

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