

Elephant Tuberculosis References (by date)
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- Dumonceaux, G.A., St. Leger, J., Olsen, J.H., Burton, M.S., Ashkin, D., and Maslow, J.N. In press. Genitourinary and pulmonary multidrug resistant *Mycobacterium tuberculosis* infection in an Asian elephant (*Elephas maximus*).
- Mikota, S.K. and Maslow, J.N. 2011. Tuberculosis at the human-animal interface: An emerging disease for elephants. *Tuberculosis* 91: 208-211.
Summary: Over the past 15 years, cases of infection with organisms of the *Mycobacterium tuberculosis* complex have been diagnosed among captive elephants in the United States and worldwide. Outbreak investigations have documented that among staff employed at facilities housing infected animals, skin test conversion to purified protein derivative have been documented. Clonal spread among animals in close contact and even inter-species spread between elephant and human has been documented. Detection of actively infected animals relies on samples obtained by trunk wash. Diagnosis has been augmented by the development of a multi-antigen serologic assay with excellent specificity and sensitivity. Treatment regimens are still in development with efficacy largely unknown due to a paucity of both premortem follow-up and necropsy data of treated animals. The epidemiology, diagnosis and treatment of tuberculosis in elephants require additional careful study of clinical data.
- Murphree, R., Warkentin, J.V., Dunn, J.R., Schaffner, W., Jones, T.F., 2011. Elephant-to-human transmission of tuberculosis, 2009. *Emerg Infect Dis* 17, 366-371.
Abstract: In 2009, the Tennessee Department of Health received reports of 5 tuberculin skin test (TST) conversions among employees of an elephant refuge and isolation of *Mycobacterium tuberculosis* from a resident elephant. To determine the extent of the outbreak and identify risk factors for TST conversion, we conducted a cohort study and onsite assessment. Risk for conversion was increased for elephant caregivers and administrative employees working in the barn housing the *M. tuberculosis*-infected elephant or in offices connected to the barn (risk ratio 20.3, 95% confidence interval 2.8-146.7). Indirect exposure to aerosolized *M. tuberculosis* and delayed or inadequate infection control practices likely contributed to transmission. The following factors are needed to reduce risk for *M. tuberculosis* transmission in the captive elephant industry: increased knowledge about *M. tuberculosis* infection in elephants, improved infection control practices, and specific occupational health programs.
- Angkawanish, T., Wajjwalku, W., Sirimalaisuwan, A., Mahasawangkul, S., Kaewsakhorn, T., Boonsri, K., Rutten, V.P.M.G., 2010. *Mycobacterium tuberculosis* infection of domesticated Asian elephants, Thailand. *Emerg Infect Dis* 16, 1949-1951.
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- Landolfi, J.A., Mikota, S.K., Chosy, J., Lyaschenko, K.P., Giri, K., Gairhe, K., Terio, K.A., 2010. Comparison of systemic cytokine levels in *Mycobacterium* spp seropositive and seronegative Asian elephants (*Elephas maximus*). *J. Zoo. Wildl. Med.* 41, 445-455.
Abstract: *Mycobacterium* spp. infection is an important health concern for Asian elephant (*Elephas maximus*) populations worldwide. The disease is of particular concern considering its potential to affect not only the individual animal but also herd and public health. Although elephant tuberculosis susceptibility is poorly understood, immune function alterations are central to disease pathogenesis in other species and probably affect outcome of mycobacterial infections in elephants. Measurement of immune mediator (cytokine) levels within blood samples can provide information regarding immune function that may elucidate disease susceptibility. For this study, mRNA levels of interleukin (IL)-2, IL-4, IL-10, and IL-12; interferon (IFN)- γ ; tumor necrosis factor (TNF)- α ; and transforming growth factor (TGF)- β were measured using elephant-specific, real-time reverse transcription-polymerase chain reaction (RT-PCR) assays in RNA-preserved whole blood samples from 106 Asian elephants, 15% of which were *Mycobacterium tuberculosis* complex seropositive. The Elephant TB STAT-PAKH (Chembio Diagnostics, Inc., Medford, New York 11763, USA), a novel lateral flow antibody detection assay

developed for specific use in elephants, was used to determine serologic status for the study. Seropositive animals had higher levels of TNF- α and lower levels of TGF- β than seronegative animals; these differences between groups were statistically significant when levels were analyzed as categorical variables. Trends toward higher levels of IFN- γ and IL-4 and slightly lower levels of IL-10 and IL-12 were noted in the seropositive group, although differences between groups were not statistically significant. Presence of other inflammatory conditions was found to be a significant confounding variable in the analysis of the relationship between tuberculosis status and TNF- α levels, necessitating its inclusion in statistical models. Age and sex were not found to significantly affect the relationship between tuberculosis status and any of the cytokines measured. Interleukin-2 levels were below the sensitivity of the realtime RT-PCR assay irrespective of tuberculosis status. These findings provide a foundation for future research into the immunopathogenesis of elephant tuberculosis.

- Michel, A.L., Muller, B., van Helden, P.D., 2010. *Mycobacterium bovis* at the animal-human interface: A problem of not? *Veterinary Microbiology* 140, 371-381.
Abstract: *Mycobacterium bovis* is a pathogen of significant importance in livestock and a wide range of wild animal species worldwide. It is also known to cause tuberculosis disease in humans, a fact which has raised renewed concerns regarding the zoonotic risk for humans, especially those living at the animal-human interface. This review consolidates recent reports in the literature mainly on animal and zoonotic tuberculosis with an emphasis on evolution, epidemiology, treatment and diagnosis. The information presented reveals the fundamental differences in the complexity and level at which the disease affects the economy, ecosystem and human population of regions where animal tuberculosis control is achieved and regions where little or no control is implemented. In conclusion the review suggests that bovine tuberculosis has essentially been reduced to a disease of economic importance in the developed world, while low-income countries are facing a multifaceted impact which potentially affects the health of livestock, humans and ecosystems and which is likely to increase in the presence of debilitating diseases such as HIV/AIDS and other factors which negatively affect human livelihoods.
- Greenwald, R., Lyashchenko, O., Esfandiari, J., Miller, M., Mikota, S., Olsen, J.H., Ball, R., Dumonceaux, G., Schmitt, D., Moller, T., Payeur, J.B., Harris, B., Sofranko, D., Waters, W.R., Lyashchenko, K.P., 2009. Highly accurate antibody assays for early and rapid detection of tuberculosis in African and Asian elephants. *Clin. Vaccine Immunol.* 16, 605-612.
Abstract: Tuberculosis (TB) in elephants is a reemerging zoonotic disease caused primarily by *Mycobacterium tuberculosis*. Current methods for screening and diagnosis rely on trunk wash culture, which has serious limitations due to low test sensitivity, slow turnaround time, and variable sample quality. Innovative and more efficient diagnostic tools are urgently needed. We describe three novel serologic techniques, the ElephantTB Stat-Pak kit, multiantigen print immunoassay, and dual-path platform VetTB test, for rapid antibody detection in elephants. The study was performed with serum samples from 236 captive African and Asian elephants from 53 different locations in the United States and Europe. The elephants were divided into three groups based on disease status and history of exposure: (i) 26 animals with culture-confirmed TB due to *M. tuberculosis* or *Mycobacterium bovis*, (ii) 63 exposed elephants from known-infected herds that had never produced a culture-positive result from trunk wash samples, and (iii) 147 elephants without clinical symptoms suggestive of TB, with consistently negative trunk wash culture results, and with no history of potential exposure to TB in the past 5 years. Elephants with culture-confirmed TB and a proportion of exposed but trunk wash culture-negative elephants produced robust antibody responses to multiple antigens of *M. tuberculosis*, with seroconversions detectable years before TB-positive cultures were obtained from trunk wash specimens. ESAT-6 and CFP10 proteins were immunodominant antigens recognized by elephant antibodies during disease. The serologic assays demonstrated 100% sensitivity and 95 to 100% specificity. Rapid and accurate antibody tests to identify infected elephants will likely allow earlier and more efficient treatment, thus limiting transmission of infection to other susceptible animals and to humans.
- Landolfi, J.A., Schultz, S.A., Mikota, S.K., Terio, K.A., 2009. Development and validation of cytokine quantitative, real time RT-PCR assays for characterization of Asian elephant immune responses 71. *Vet. Immunol. Immunopathol.* 131, 73-78.
Abstract: Infectious disease is an important factor in Asian elephant health and long-term species survival. In studying disease pathogenesis, it is important to consider not only the pathogen, but also the effectiveness of the host immune response. Currently, there is a paucity of information available on elephant immune function. Measurement of cytokine levels within clinical samples can provide valuable information regarding immune function during health and disease that may elucidate disease susceptibility. To develop tools for assessment of

elephant immune function, Asian elephant partial mRNA sequences for interleukin (IL)-2, IL-4, IL-10, IL-12, interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha, transforming growth factor (TGF)-beta, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and beta-actin were determined. Sequence information was then utilized to design elephant-specific primers and probes for quantitative, real time, RT-PCR assays for the measurement of cytokine mRNA. Greater than 300bps of Asian elephant mRNA sequence were determined for each cytokine of interest. Consistent and reproducible, real time, RT-PCR assays with efficiencies of greater than 93% were also developed. Assay sensitivities ranged from less than 1 to 5000 DNA copies with the exception of IL-12, which had a sensitivity of 42,200 copies. Employment of molecular techniques utilizing mRNA-based detection systems, such as real time, RT-PCR, facilitate sensitive and specific cytokine detection and measurement in samples from species for which commercial reagents are not available. Future studies utilizing these techniques to compare elephant immune function during health and in the face of infection will be useful for characterizing the contribution of the elephant immune system to disease

- Michel, A.L., Coetzee, M.L., Keet, D.F., Mare, L., Warren, R., Cooper, D., Bengis, R.G., Kremer, K., van Helden, P., 2009. Molecular epidemiology of *Mycobacterium bovis* isoaltes from free-ranging wildlife in South African game reserves. *Vet Microbiol* 133, 335-343.
Abstract: Bovine tuberculosis is endemic in African buffalo and a number of other wildlife species in the Kruger National Park (KNP) and Hluhluwe-iMfolozi Park (HiP) in South Africa. It was thought that the infection had been introduced into the KNP ecosystem through direct contact between cattle and buffalo, a hypothesis which was confirmed in this study by IS6110 and PGRS restriction fragment length polymorphism (RFLP) typing. The molecular characterisation of 189 *Mycobacterium bovis* isolates from nine wildlife species in the HiP, including three smaller associated parks, and the Kruger National Park with adjacent areas showed that the respective epidemics were each caused by an infiltration of a single *M.bovis* genotype. The two *M. bovis* strains had different genetic profiles, as demonstrated by hybridisation with the IS6110 and PGRS RFLP probes, as well as with regard to evidence of evolutionary changes to the IS profile. While the *M. bovis* type in HiP was transmitted between buffaloes and to at least baboon, bushpig and lion without obvious genetic changes in the RFLP patterns, in the KNP a dominant strain was represented in 73% of the *M. bovis* isolates, whilst the remaining 27% were variants of this strain. No species-specific variants were observed, except for one IS6110 type which was found only in a group of five epidemiologically related greater kudu. This finding was attributed to species-specific behaviour patterns rather than an advanced host-pathogen interaction.
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Ref Type: Conference Proceeding
Abstract: In many domestic species, routine hematology assays are useful diagnostic tools to diagnose inflammatory conditions. Unlike other species, these hematologic tests apparently are insensitive indicators of inflammation in elephants.1 We studied a novel group of blood proteins, called acute phase proteins, which increase during inflammatory conditions, for their usefulness in diagnosing elephants with inflammatory diseases. Although these proteins currently are useful in humans and domestic animals, each species has a different set of important proteins that must be individually investigated.2 We tested several acute phase proteins (C-reactive protein, alpha-1 glycoprotein, alpha-1 antitrypsin, serum amyloid A, haptoglobin, fibrinogen, ceruloplasmin, and albumin) as well as complete blood counts, chemistry panels, serum protein electrophoresis, and 3-D gel electrophoresis to determine their usefulness for diagnosing different types of inflammatory conditions in Asian elephants (*Elephas maximus*). Animals with inflammatory conditions were classified as those individuals with known illnesses such as mycobacteriosis, arthritis, nail bed abscesses, and malignant tumors. Control animals were those animals that were suspected to not have any inflammation and be healthy at the time of testing as determined by physical examination and obtaining a thorough medical history.

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Abstract: Transmission of tuberculosis occurs with the highest frequency from patients with extensive, cavitary, pulmonary disease and positive sputum smear microscopy. In animal models of tuberculosis, the development of caseous necrosis is an important prerequisite for the formation of cavities although the immunological triggers for liquefaction are unknown. We review the relative merits and the information gleaned from the available animal models of pulmonary cavitation. Understanding the host-pathogen interaction important to the formation of cavities may lead to new strategies to prevent cavitation and thereby, block transmission.
- 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*). J. Zoo. Wildl. Med. 38, 101-107.
Abstract: *Mycobacterium szulgai* was associated with mortality in two captive African elephants (*Loxodonta africana*) housed at Lincoln Park Zoo. The first elephant presented with severe, acute lameness of the left rear limb. Despite extensive treatments, the animal collapsed and died 13 mo after initial presentation. Necropsy revealed osteomyelitis with loss of the femoral head and acetabulum and pulmonary granulomas with intralesional *M. szulgai*. The second elephant collapsed during transport to another institution with no premonitory clinical signs. This animal was euthanized because of prolonged recumbency. Granulomatous pneumonia with intralesional *M. szulgai* was found at necropsy. Two novel immunoassays performed on banked serum samples detected antibody responses to mycobacterial antigens in both infected elephants. It was not possible to determine when the infection was established or how the elephants were infected. When reviewing the epidemiology of this organism in humans, however, transmission between elephants seemed unlikely because human-to-human transmission of this organism has never been reported and a third elephant in the herd was not affected. In addition to *Mycobacterium bovis* and *Mycobacterium tuberculosis*, atypical mycobacterial organisms need to be considered potentially pathogenic in elephants.
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Abstract: Tuberculosis (TB) caused by Mycobacterial organisms has emerged as one of the major diseases in captive elephants. In vitro Interferon-gamma (IFN-gamma) assay is being used as an ancillary test for early detection of TB in domestic and captive wild animals. In the present study, basic sequence information and immunological cross-reactivity of this major cytokine of Asian elephants were explored. At predicted amino acid level, IFN-gamma of Asian elephant showed maximum identity to that of horse (73%). Other IFN-gamma amino acid sequences that showed high level identity were that of giant panda (72%), dog (71%), nine-banded armadillo (69%), cattle (63%) and human (62%). IFN-gamma promoter sequences of Asian elephant, human, cattle and mouse showed high level conservation of the putative transcription factor binding sites, TATA box and transcriptional start site. The functionally important human IFN-gamma promoter elements, such as AP-2/IRF-3, YY1-gammaIFN-BED, ATFCS and AP-1/gammaINF binding sites, were absolutely conserved in the corresponding elephant sequence. There was only a single nucleotide variation in the other two important elements, NFAT-gammaINF and IFN-gammaPE, indicating the highly conserved regulation of IFN-gamma expression across different species. Phylogenetic analysis based on IFN-gamma protein sequences revealed a closer relation of Asian elephants and nine-banded armadillo. This shows a closer evolution of these members of Afrotheria and Xenarthra, respectively; and supports the previous reports based on mitochondrial DNA studies. In Western blot analysis, IFN-gamma of Asian elephant expressed in *Escherichia coli* was detected using an anti-bovine IFN-gamma monoclonal antibody, indicating immunological cross-reactivity

- Une, Y., Mori, T., 2007. Tuberculosis as a zoonosis from a veterinary perspective. *Comp Immunol Microbiol Infect Dis* 30, 415-425.
Abstract: Tuberculosis is an important disease among many zoonoses, because both *Mycobacterium tuberculosis* and *Mycobacterium bovis*, which are the major causes of tuberculosis, are highly pathogenic, infect many animal species and thus are likely to be the source of infection in humans. In particular, monkeys are highly susceptible to these bacteria and are important spreaders. Recently, two outbreaks of *M. tuberculosis* occurred in four different kinds of monkeys and humans were also infected with the disease in Japan. In zoos, tuberculosis was reported not only in monkeys, but also in several different kinds of animals, including elephants. Pets such as dogs and cats are believed to be generally less susceptible to *M. tuberculosis*, but in this article we introduce a case of infection from man to dog by close contact. Japan is one of the few countries that have been able to control *M. bovis* infection. In other countries, however, cases of bovine tuberculosis and human *M. bovis* infection have been reported, and thus further attention is still required in the future.
- Ball, R., Dumonceaux, G., Olsen, J., Burton, M.S., 2006. Comparison of trunk wash results matched to Multiantigen Print Immunoassay (MAPIA) in a group of captive Asian elephants (*Elephas maximus*). *Proceedings International Elephant Conservation and Research Symposium* 242-243.
- Ball, R.L., Dumonceaux, G., Olsen, J.H., Burton, M.S., Lyashchenko, K. Comparison of trunk wash results matched to multiantigen print immunoassay (MAPIA) in a group of captive Asian elephants (*Elephas maximus*). 2006 *Proceedings American Association of Zoo Veterinarians*. 303-304. 2006.
Abstract: Introduction: Between 1994 and June 2005, there were 34 confirmed cases of tuberculosis in elephants in the U.S. population. Thirty-one Asian (*Elephas maximus*) and three African (*Loxodonta africana*) elephants were affected. *Mycobacterium tuberculosis* was the etiologic agent in 33 cases and *M. bovis* in one case. Cases of tuberculosis caused by an unusual nontuberculous mycobacteria, *M. szulgai* have recently occurred as well. Currently, TB in elephants remains a diagnostic dilemma. The sensitivity of trunk wash culture, the currently recommended test for diagnosis, is unknown. False negatives have been documented (trunk wash negative elephants that were subsequently found to be culture positive at necropsy). Other non-culture techniques for TB diagnosis include ELISA, and PCR. A novel technology, MultiAntigen Print ImmunoAssay (MAPIA) and lateral-flow technology (Rapid Test) has been evaluated and used to diagnose tuberculosis in captive elephants with encouraging results. One concern with this serologic testing is the possibility of *Mycobacterium* other than tuberculosis (MOTT) cross-reacting with the antigen used in the Rapid Test or the MAPIA and leading to a false positive. With numerous MOTT routinely cultured from trunk washes, this is a valid concern. Methods and Materials: A retrospective analysis was done at Busch Gardens Tampa Bay and Chembio, Inc. that matched trunk wash results to serum samples. All serum was collected within 7 days of the trunk wash and analyzed with the Rapid Test and MAPIA. Four Asian elephants with a total of 18 samples met this criteria and had serum submitted for testing. Results and Discussion: Table 1 lists the results and the organisms cultured. While the sampling is limited in this pilot project, it appears that MOTT does not evoke a response when assayed with the Rapid Test or MAPIA. The recent cases of *M. szulgai* do demonstrate the potential usefulness for this test when a disease develops from MOTT. The usefulness of this new technology, taken in conjunction with other clinical data including trunk washes when indicated, is a valuable tool in the healthcare of captive elephants.

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Abstract: Transmission of tuberculosis occurs with the highest frequency from patients with extensive, cavitary, pulmonary disease and positive sputum smear microscopy. In animal models of tuberculosis, the development of caseous necrosis is an important prerequisite for the formation of cavities although the immunological triggers for liquefaction are unknown. We review the relative merits and the information gleaned from the available animal models of pulmonary cavitation. Understanding the host-pathogen interaction important to the formation of cavities may lead to new strategies to prevent cavitation and thereby, block transmission
- 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. *Clin. Vaccine Immunol.* 13, 722-732.

Abstract: Tuberculosis (TB) in elephants is a re-emerging zoonotic disease caused primarily by *Mycobacterium tuberculosis*. Current diagnosis relies on trunk wash culture, the only officially recognized test, which has serious limitations. Innovative and efficient diagnostic methods are urgently needed. Rapid identification of infected animals is a crucial prerequisite for more effective control of TB, as early diagnosis allows timely initiation of chemotherapy. Serology has diagnostic potential, although key antigens have not been identified and optimal immunoassay formats are not established. To characterize the humoral responses in elephant TB, we tested 143 serum samples collected from 15 elephants over time. These included 48 samples from five culture-confirmed TB cases, of which four were in Asian elephants infected with *M. tuberculosis* and one was in an African elephant with *Mycobacterium bovis*. Multiantigen print immunoassay (MAPIA) employing a panel of 12 defined antigens was used to identify serologic correlates of active disease. ESAT-6 was the immunodominant antigen recognized in elephant TB. Serum immunoglobulin G antibodies to ESAT-6 and other proteins were detected up to 3.5 years prior to culture of *M. tuberculosis* from trunk washes. Antibody levels to certain antigens gradually decreased in response to antitubercular therapy, suggesting the possibility of treatment monitoring. In addition to MAPIA, serum samples were evaluated with a recently developed rapid test (RT) based on lateral flow technology (ElephantTB STAT-PAK). Similarly to MAPIA, infected elephants were identified using the RT up to 4 years prior to positive culture. These findings demonstrate the potential for TB surveillance and treatment monitoring using the RT and MAPIA, respectively
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Abstract: Tuberculosis, caused by *Mycobacterium bovis*, was first diagnosed in African buffalo in South Africa's Kruger National Park in 1990. Over the past 15 years the disease has spread northwards leaving only the most northern buffalo herds unaffected. Evidence suggests that 10 other small and large mammalian species, including large predators, are spillover hosts. Wildlife tuberculosis has also been diagnosed in several adjacent private game reserves and in the Hluhluwe-iMfolozi Park, the third largest game reserve in South Africa. The tuberculosis epidemic has a number of implications, for which the full effect of some might only be seen in the longterm. Potential negative long-term effects on the population dynamics of certain social animal species and the direct threat for the survival of endangered species pose particular problems for wildlife conservationists. On the other hand, the risk of spillover infection to neighboring communal cattle raises concerns about human health at the wildlife-livestock-human interface, not only along the western boundary of Kruger National Park, but also with regards to the joint development of the Greater Limpopo Transfrontier Conservation Area with Zimbabwe and Mozambique. From an economic point of view, wildlife tuberculosis has resulted in national and international trade restrictions for affected species. The lack of diagnostic tools for most species and the absence of an effective vaccine make it currently impossible to contain and control this disease within an infected free-ranging ecosystem. Veterinary researchers and policy-makers have recognized the need to intensify research on this disease and the need to develop tools for control, initially targeting buffalo and lion.
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Abstract: Serologic tests including the ELISA, MAPIA (Multi-Antigen Print Immunoassay), and a rapid test, VetTB StatPak® (Chembio Diagnostic Systems, Inc., Medford, New York 11763 USA) have recently been

developed and show great promise for the diagnosis of tuberculosis (TB) in elephants. These serologic tests detect antibodies to antigens of *Mycobacterium tuberculosis* complex organisms and in some cases have detected infection years in advance of active disease and mycobacterial shedding. The diagnosis of active TB (by culture) or serologic conversion presents management challenges for captive elephants in Asian range countries. Of the 2 billion humans world-wide infected with TB, fewer than 10% will develop active disease. This figure is unknown for elephants. The identification and management of infected elephants has ramifications for elephants and humans alike and issues such as public health and tourism may be impacted. TB is endemic among humans in Asia and where there is intermingling of elephants and humans, both species may act as reservoirs for disease transmission. The various situations in which elephants are kept in Asia (government-owned, privately-owned, festivals, temples, zoos, etc.) make it difficult to develop a management strategy that will address all circumstances. Other concerns are the cost of treatment for an elephant (~ \$50,000 USD) and appropriate monitoring in resource-poor countries. The authors have recently undertaken the screening of 120 elephants in Nepal to further evaluate the above-mentioned (and other) diagnostic tests. To our knowledge, this is the first organized, large-scale initiative to screen Asian elephants within a range country. Preliminary discussions regarding the management of both culture and serologically positive government-owned and privately-owned elephants in Nepal have been initiated and may serve as a starting point for other countries as more elephants are screened within Asia. Basic options for active (culture-positive) cases include (1) treatment, (2) segregation or (3) euthanasia. Options for latent disease (culture-negative, serologically positive) cases include (1) treatment, (2) segregation and monitoring for active disease and (3) euthanasia. The particular ownership/husbandry system, available resources and cultural constraints may dictate final management choices in range countries.

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 Abstract: Mycolic acids and structures attached to them constitute a major part of the protective envelope of *Mycobacterium tuberculosis*, and for this reason, their role in tuberculosis pathogenesis has been extensively studied. In this issue of the JCI, Rao et al. examine the effect of trans-cyclopropanation of oxygenated mycolic acids attached to trehalose dimycolate (TDM) on the murine immune response to infection (see the related article beginning on page 1660). Surprisingly, they found that an *M. tuberculosis* mutant lacking trans-cyclopropane rings was hypervirulent in mice. The recent recognition of a hypervirulence phenotype in mice associated with laboratory and clinical *M. tuberculosis* strains with altered cell wall components has provided new insights into how *M. tuberculosis* may establish persistent infection. However, to date, characterization of these bioactive products in pathogenesis has been largely reductionistic; the relationship of their effects observed in mice to the persistent infection and tuberculosis caused by *M. tuberculosis* observed in humans remains obscure
- Rothschild, B.M., Martin, L.D., 2006. Did ice-age bovids spread tuberculosis? *Naturwissenschaften* 93, 565-569.
 Abstract: Pathognomonic metacarpal undermining is a skeletal pathology that has been associated with *Mycobacterium tuberculosis* in bovids. Postcranial artiodactyl, perissodactyl, and carnivore skeletons were examined in major university and museum collections of North America and Europe for evidence of this and other pathology potentially attributable to tuberculosis. Among nonproboscidean mammals from pre-Holocene North America, bone lesions indicative of tuberculosis were restricted to immigrant bovids from Eurasia. No bone lesions compatible with diagnosis of tuberculosis were found in large samples of other pre-Holocene (164 Oligocene, 397 Miocene, and 1,041 Plio-Pleistocene) North American mammals, including 114 antilocaprids. Given the unchanged frequency of bovid tubercular disease during the Pleistocene, it appears

that most did not die from the disease but actually reached an accommodation with it (as did the mastodon) (Rothschild and Laub 2006). Thus, they were sufficiently long-lived to assure greater spread of the disease. The relationships of the proboscidean examples need further study, but present evidence suggests a Holarctic spread of tuberculosis during the Pleistocene, with bovids acting as vectors. While the role of other animals in the transmission of tuberculosis could be considered, the unique accommodation achieved by bovids and mastodons makes them the likely "culprits" in its spread.

- Rothschild, B.M., Laub, R., 2006. Hyperdisease in the late Pleistocene: validation of an early 20th century hypothesis. *Naturwissenschaften* 93, 557-564.
- Cousins, D.V., Florisson, N., 2005. A review of tests available for use in the diagnosis of tuberculosis in non-bovine species. *Rev. sci. tech. Off. int. Epiz.* 24, 1039-1059.
Abstract: Bovine tuberculosis is an important disease that has impacts on regional and international trade. The disease can affect both social and economic stability and have a deleterious affect on species diversity. The intradermal tuberculin test has been in use for almost a century and, despite the technological advances of the last two decades, is still the only prescribed test for the diagnosis of tuberculosis in cattle. Many other species of animal, including humans, can be infected with *Mycobacterium bovis*. This paper reviews the various tests that have been used by researchers for detecting infection with *M. bovis* in a variety of animal species, and attempts to prioritise or comment on the importance of having appropriately validated diagnostics for the different species. The difficulties of test validation using small numbers of animals, especially when tuberculosis occurs in only a few instances or the species of animal affected is rare and/or valuable, are discussed.
- Lacasse, C., Gamble, K.C., Terio, K., Farina, L.L., Travis, D.A., Miller, M. *Mycobacterium szulgai* osteoarthritis and pneumonia in an African elephant (*Loxodonta Africana*). 2005 Proceedings AAZV, AAWV, AZA Nutrition Advisory Group. 170-172. 2005.
Abstract: Tuberculosis, particularly *Mycobacterium bovis* and *M. tuberculosis*, is an important health issue in zoological collections. Zoos are a particular public health concern because of the close contact between tuberculosis-susceptible animals and humans, specifically animal handlers and visitors.¹⁶ Evidence of *M. tuberculosis* transmission between humans and elephants, confirmed by DNA fingerprinting, has been reported.¹³ Between 1994 and 2001, *M. tuberculosis* was isolated from trunk washes of captive elephants from 11 herds in the United States.¹⁷ To date, most reported cases of tuberculosis have occurred in captive Asian elephants (*Elephas maximus*).¹⁴ In 1997, the National Tuberculosis Working Group for Zoo and Wildlife Species partnered with the USDA to formulate the "Guidelines for the Control of Tuberculosis in Elephants."¹⁵ This document outlines criteria for the testing, surveillance, and treatment of tuberculosis in elephants. The guidelines recommend annual monitoring of elephants by mycobacterial culture of three direct trunk washes collected over 1 wk. Isolation of *Mycobacterium avium* and non-tuberculous mycobacteria from elephant trunk wash samples is common, but these organisms have not been associated with clinical disease.^{14,18} This case report details clinical disease with fatal complications of an atypical mycobacterial infection in an African elephant (*Loxodonta africana*). In September 2003, an African elephant presented with acute, severe lameness of the left rear limb with subsequent swelling of the stifle. Diagnostic procedures included aspiration cytology of the swelling, radiographs, and thermographic imaging. The exact location of the injury could not be detected, but a lesion to the stifle or coxofemoral articulation was suspected. After 13 mo of treatment, including pulse therapy with a variety of nonsteroidal anti-inflammatory drugs (NSAIDs), weekly to biweekly injections of polysulfated glycosaminoglycan, and intensive foot care efforts to treat secondary pedal lesions of both rearlimbs, the animal died acutely. Gross necropsy revealed granulomatous osteomyelitis with necrosis/loss of the femoral head and acetabulum and pulmonary granulomas. Both of these lesions contained acid-fast bacteria on cytology. While awaiting confirmatory culture results, quarantine procedures were established for the elephant facility and a program was established to screen all zoo personnel in close contact with the elephant or who participated in the necropsy. All personnel were tested by the Chicago Department of Public Health without documented conversion. *Mycobacterium szulgai* was ultimately cultured from both coxofemoral and pulmonary lesions. *Mycobacterium szulgai* is an uncommon nontuberculous mycobacterium that is usually isolated from pathologic lesions in humans.²¹ This bacterial species was first identified in 1972.¹¹ The lungs are the main locality for pathologic manifestation in humans and several cases have been in patients with acquired immunodeficiency syndrome.^{9,20,21} Infection due to *M. szulgai* most frequently produces thin-walled cavities in lungs resembling tuberculosis.⁴ Other documented sites of infection include the skin, bone, and tendon sheath (causing a carpal

tunnel syndrome).^{2,9,10,12,19,20} Intra-operative contamination from ice water has led to *M. szulgai* keratitis after laser-assisted ophthalmic surgeries.⁶ A case of disseminated disease in a previously healthy young human has been reported.⁵ No evidence of human-to-human transmission of this organism has been documented and human cases are believed to originate from environmental sources.¹² The natural habitat of the organism is unknown, but previous reports suggest an association of the bacteria with water of swimming pools and fish tanks.^{1,21} The organism has been cultured from a snail and tropical fish.^{1,3} No standard recommendation for the treatment of *M. szulgai* infection currently exists. In general, triple antibiotic therapies used in standard mycobacterial treatments are reported with a low rate of relapses and sterilization of sputum cultures within a mean of 3 mo.³ Pulmonary lesions in this elephant were chronic; it was not possible to determine when initial infection occurred. Infection could have occurred in captivity or in the wild prior to captivity. Three trunk washes over the past year had been negative for mycobacterial culture. Osteomyelitis in the hip may have developed secondary to hematogenous spread from the lungs with the acute lameness resulting from a pathologic fracture associated with this infection. Alternatively, though considered less likely, a traumatic fracture of the hip could have occurred, with bacterial inoculation and secondary osteomyelitis as a result of increased blood flow to the site. The source of infection for this elephant remains unknown. Prevalence of this organism in the natural habitat or captive environment of the elephants has not been previously documented.

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- Larsen, R.S., Kay, M., Triantis, J., Salman, M.D. Update on serological detection of *Mycobacterium tuberculosis* infection in Asian elephants. 2005 Proceedings AAZV, AAWV, AZA Nutrition Advisory Group. 62-63. 2005. Abstract: Tuberculosis has become an important disease in captive elephants, particularly Asian elephants (*Elephas maximus*). Diagnosing tuberculosis in elephants has been problematic as many tests have inadequate sensitivity or specificity. 2-4 A multiple-antigen enzyme-linked immunosorbent assay (ELISA) was previously investigated for detecting infection in Asian elephants and African elephants (*Loxodonta africana*); this test had excellent sensitivity and specificity, but needed further evaluation. 1 Modifications to the multiple-antigen ELISA panel have since been made. Valuable antigens were retained, other antigens were removed, and new ones were added. This modified ELISA was re-evaluated, using serum from 68 Asian elephants. Sixteen had *M. tuberculosis* -positive trunk cultures, while 52 were either culture negative at necropsy or had a history of negative trunk cultures and no contact with infected elephants. Seven elephants were evaluated over time. The test was 100% (95% CI; 95-100%) specific and 94% (95% CI; 79-100%) sensitive using two of the six antigens (*M. bovis* strain AN5 culture filtrate and *M. tuberculosis* early secretory antigenic target 6). "Effectively-treated" elephants had decreasing seroreactivity, but those that were culture-positive post-treatment were more consistently seroreactive. Although "effectively-treated" elephants had declining seroreactivity, they still usually had higher values than animals that had never been infected. Serology continues to show great promise in detecting tuberculosis in elephants, often detecting infection months-to-years sooner than trunk wash culture. Advances in techniques may soon make serology even more practical. While serology should not replace trunk-wash culture, it is a useful adjunct for early detection of infection in elephants and for monitoring treatment.

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- 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. Vet. Rec. 156, 171-175. Abstract: Between 2001 and 2003, there was an outbreak of tuberculosis in a Swedish zoo which involved elephants, giraffes, rhinoceroses and buffaloes. Cultures of trunk lavages were used to detect infected elephants, tuberculin testing was used in the giraffes and buffaloes, and tracheal lavage and tuberculin testing were used in the rhinoceroses. The bacteria isolated were investigated by spoligotyping and restriction fragment length polymorphism. Five elephants and one giraffe were found to have been infected by four different strains of *Mycobacterium tuberculosis*.

- Lyashchenko, K., Miller, M., Waters, W.R. Application of MAPIA (Multiple antigen print immunoassay) and rapid lateral flow technology for tuberculosis testing of elephants. 2005 Proceedings AAZV, AAWV, AZA Nutrition Advisory Group. 64-65. 2005.
Abstract: Tuberculosis (TB) remains a serious re-emerging disease in wildlife and zoo animals. *Mycobacterium tuberculosis* has been isolated from 30 captive Asian elephant (*Elephas maximus* within 14 herds in the United States (1994-2004) and *Mycobacterium bovis* has been isolated from one African elephant (*Loxodonta africana*) (Mikota, pers. comm.).³ There are several challenges with elephant TB diagnosis. Culture of trunk wash has relatively poor sensitivity and is subject to contamination. Skin test is not validated in elephants and there is little reliability in these results.⁴ Serologic tests are appealing because samples can be stored for future analysis, archived samples can be analyzed, various assay platforms can be directly compared, and these assays are amenable to serial analysis (e.g., to monitor therapy). There is currently a multiple antigen ELISA test available for experimental use in elephants.¹

To improve tuberculosis control, new diagnostic tools should be rapid, accurate, and host species-independent. Two novel serologic methods, MultiAntigen Print ImmunoAssay (MAPIA) and lateral-flow technology (Rapid Test), have been adapted for use in white-tailed deer, European badger, cattle, and Asian and African elephants for the detection of TB-specific antibody. Serologic markers of diagnostic importance have been identified for each host tested so far. With MAPIA, a machine prints specific antigens horizontally on a nitrocellulose membrane which can be cut into strips and used in Western blot.² Strips are incubated with test serum samples, then an anti-Ig conjugate and color developer. Using this assay, an antibody response to multiple mycobacterial antigens has been observed in sera from *M. tb*-infected elephants. No antibody response was detected to any antigens in non-infected elephant sera. Additionally, the kinetics of antibody responses by elephants undergoing antibiotic therapy indicates that the MAPIA could be used for monitoring treatment and to determine recrudescence of infection.

Using selected antigens, a lateral-flow test was developed for rapid antibody detection that can be used in multiple species. The Rapid Test can use serum, plasma, or whole blood and provides results within 15 min. These tests are similar to in-clinic tests for FIV/FeLV detection (snap test, IDDEX). If a band is present in the test strip, it indicates a positive reaction (antibody present).

A panel of sera from healthy and TB infected elephants showed good correlation between the MAPIA and the rapid test (Table 1).

In summary, it appears that TB-infected elephants produce a robust antibody response that can be detected in serologic assays. Of special significance is the kinetics of the response, which may permit earlier detection of infection than current diagnostic methods. While initial results are promising, additional studies are required to validate these two assays. A relatively small set of serum samples from documented infected and non-infected elephants was used, and more samples are needed to further validate the tests. MAPIA has been used to optimize antigen selection in order to make the most sensitive and specific Rapid Test. This strategy may also allow for identification of "treatment-sensitive" antigens that could be used in the MAPIA format to monitor TB therapy. While elephants will be used as an initial "proof of concept" species for test development, additional samples from other species will also be evaluated to determine applicability to other species (i.e., a host species-independent test), thus benefiting other groups such as primates, rhinos, cervids, etc.

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Abstract: We recently described the clinical presentation and treatment of 18 elephants from six herds infected with TB. Treatment protocols and methods varied between herds to include both oral and rectal dosing using multiple drug doses and formulations. In this paper we present information regarding the pharmacokinetics (PK) of isoniazid (INH) in elephants and provide suggestions regarding initial treatment regimens. Forty-one elephants received INH daily by either oral or rectal administration with different formulations. Population PK analysis was performed using Non-linear Mixed Effect Modeling (NONMEM). Results of oral administration indicated that compared with premixed INH solution, the drug exposure was highest with a suspension prepared freshly with INH powder. When INH was concomitantly given as an admixture over food, T_{max} was delayed and variability in drug absorption was significantly increased. Compared with oral administration, similar drug exposures were found when INH was dosed rectally. The data generated suggest that a starting dose of 7.5 mg/kg of INH is appropriate for initial TB treatment in elephants when premixed solution is administered directly into the oropharynx or rectal vault and 4 mg/kg are when INH is administered following immediate suspension from powdered form
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Abstract: In this comparative study, we have established in vitro models of equine and elephant articular chondrocytes, examined their basic morphology, and characterized the biophysical properties of their primary voltage-gated potassium channel (K_v) currents. Using whole cell patch-clamp electrophysiological recording from first-expansion and first-passage cells, we measured a maximum K_v conductance of 0.15 +/- 0.04 pS/pF (n = 10) in equine chondrocytes, whereas that in elephant chondrocytes was significantly larger (0.8 +/- 0.4 pS/pF, n = 4, P <= 0.05). Steady-state activation parameters of elephant chondrocytes (V = -22 +/- 6 mV, k = 11.8 +/- 3 mV, n = 4) were not significantly different from those of horse chondrocytes (V = -12.5 +/- 4.3 mV, k = 12 +/- 2, n = 10). This suggests that there would be slightly more resting K_v activation in elephant chondrocytes than in their equine counterparts. Kinetic analysis revealed that both horse and elephant chondrocyte K_v currents had similar activation and inactivation parameters. Pharmacological investigation of equine chondrocyte K_v currents showed them to be powerfully inhibited by the potassium channel blockers tetraethylammonium and 4-aminopyridine but not by dendrotoxin-I. Immunohistochemical studies using polyclonal antibodies to Kv1.1-Kv1.5 provided evidence for expression of Kv1.4 in equine chondrocytes. This is the first electrophysiological study of equine or elephant chondrocytes. The data support the notion that voltage-gated potassium channels play an important role in regulating the membrane potential of articular chondrocytes and will prove useful in future modeling of electromechanotransduction of fully differentiated articular chondrocytes in these and other species
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Abstract: Despite having a very low incidence of disease, reindeer (*Rangifer tarandus*) are subject to tuberculosis (TB) testing requirements for interstate shipment and herd accreditation in the United States. Improved TB tests are desperately needed, as many reindeer are falsely classified as reactors by current testing procedures. Sera collected sequentially from 11 (experimentally) *Mycobacterium bovis*-infected reindeer and 4 noninfected

reindeer were evaluated by enzyme-linked immunosorbent assay (ELISA), immunoblotting, and multiantigen print immunoassay (MAPIA) for antibody specific to *M. bovis* antigens. Specific antibody was detected as early as 4 weeks after challenge with *M. bovis*. By MAPIA, sera were tested with 12 native and recombinant antigens, which were used to coat nitrocellulose. All *M. bovis*-infected reindeer developed responses to MPB83 and a fusion protein, Acr1/MPB83, and 9/11 had responses to MPB70. Other antigens less commonly recognized included MPB59, ESAT-6, and CFP10. Administration of purified protein derivatives for skin testing boosted serum antibody responses, as detected by each of the assays. Of the noninfected reindeer, 2/4 had responses that were detectable immediately following skin testing, which correlated with pathological findings (i.e., presence of granulomatous lesions yet the absence of acid-fast bacteria). The levels of specific antibody produced by infected reindeer appeared to be associated with disease progression but not with cell-mediated immunity. These findings indicate that *M. bovis* infection of reindeer elicits an antibody response to multiple antigens that can be boosted by skin testing. Serological tests using carefully selected specific antigens have potential for early detection of infections in reindeer.

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Abstract: This study was undertaken to characterize the population pharmacokinetics (PK), therapeutic dose, and preferred route of administration for pyrazinamide (PZA) in elephants. Twenty-three African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants infected with or in contact with others culture positive for *Mycobacterium tuberculosis* were dosed under treatment conditions. PZA was dosed daily at 20-30 mg/kg via oral (fasting or nonfasting state) or rectal (enema or suppository) administration. Blood samples were collected 0-24 h postdose. Population PK was estimated using nonlinear mixed effect modeling. Drug absorption was rapid with T(max) at or before 2 h regardless of the method of drug administration. C(max) at a mean dose of 25.6 (+/- 4.6) mg/kg was 19.6 (+/- 9.5 microg/mL) for PZA given orally under fasting conditions. Under nonfasting conditions at a mean dose of 26.1 +/- 4.2 mg/kg, C(max) was 25% (4.87 +/- 4.89 microg/mL) and area under concentration curve (AUC) was 30% of the values observed under fasting conditions. Mean rectal dose of 32.6 +/- 15.2 mg/kg yielded C(max) of 12.3 +/- 6.3 microg/mL, but comparable AUC to PZA administered orally while fasting. Both oral and rectal administration of PZA appeared to be acceptable and oral dosing is preferred because of the higher C(max) and lower inter-subject variability. A starting dose of 30 mg/kg is recommended with drug monitoring between 1 and 2 h postdose. Higher doses may be required if the achieved C(max) values are below the recommended 20-50 microg/mL range
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- Janssen, D.L., Oosterhuis, J.E., Fuller, J., Williams, K. Field technique: A method for obtaining trunk wash mycobacterial cultures in anesthetized free-ranging African elephants (*Loxodonta africana*). 2004 PROCEEDINGS AAZV, AAWV, WDA JOINT CONFERENCE. 582-583. 2004.
Abstract: The *Guidelines for the Control of Tuberculosis in Elephants* 2003 (*Guidelines*) of the National tuberculosis Working Group for Zoo and Wildlife Species were written to protect the health and safety of captive elephants together with their handlers and the viewing public.1 The *Guidelines* specifically address the display and transport of captive elephants but do not address the unique situation of free-living elephants being imported and subsequently displayed to the public.

Although the *Guidelines* describe a technique for collecting and handling a trunk wash in a trained, standing, non-anesthetized elephant, it does not describe a similar technique for anesthetized elephants in lateral recumbency. In an attempt to detect active mycobacterial infection in a group of 3 male and 8 female free-ranging African elephants scheduled for import into the United States, a technique was developed for collecting trunk washes in recumbent, anesthetized elephants for mycobacterial culture.

A South African game-capture crew, experienced in translocating elephants, anesthetized elephants in groups via remote drug delivery and from a helicopter. The ground crew accomplished multiple simultaneous procedures including anesthesia maintenance and monitoring, physical and reproductive examinations, collection of general diagnostic and investigative samples, and trunk washes for mycobacterial cultures. This was accomplished while the capture crew was preparing animals for loading into specially designed trailers for transport to a holding boma. Little time was available for any one of procedure with multiple

animals being attended to at one time.

Once an elephant was stable in lateral recumbency, a 3-m foal stomach tube, prepackaged and sterilized, was inserted into the dependent side of the trunk tip. It was then gently fed up the trunk approximately 2.5 m. A 50-ml sample suction trap was attached to the end of the foal tube. The suction trap was then attached to a battery powered, portable aspirator pump designed for emergency medical care. The aspiration pump was activated to collect secretions from the most proximal portion of the trunk. If little or no secretions were collected by this means, the system was disconnected between the sample trap and the foal tube. Then, 100 ml of sterile saline was placed into raised end of the foal tube allowing it to drain toward the tip through gravity. The suction trap and aspiration pump were reattached to collect a sample in the sample trap. Then, the sample trap was replaced with a new trap, and the foal tube was inserted into the oral pharynx for collection of a separate oropharyngeal sample. This same procedure was repeated with each elephant.

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Abstract: From 1997 to 2000, six cases of *Mycobacterium tuberculosis* (TB) infection were diagnosed in three species of animals at, or recently originating from, the Los Angeles Zoo. Restriction fragment length polymorphism (RFLP) analysis showed that five of six animal isolates shared an identical IS6110 pattern, with the sixth differing only by one additional band. A multiinstitutional epidemiologic investigation was conducted to identify and interrupt possible transmission among the animal cases, and to screen personnel for active TB infection and TB skin-test conversion.

Animal Cases

In April and October of 1994, Asian elephant (*Elephas maximus*) #1 and Asian elephant #2 arrived at the Los Angeles Zoo from a private elephant facility where they had lived together. They were housed together at the zoo until November of 1996 when elephant #2 was returned to the facility for several months before transfer to another zoo. In the spring of 1997, Elephant #1 (30 yr old) died of salmonellosis, with *M. tuberculosis* found in granulomatous lymph node lesions from the thoracic and abdominal cavities, and Elephant #2 (30 yr old) was found to have a positive trunk wash culture for *M. tuberculosis*. In July of 1998, one of a closed herd of three Rocky Mountain goats (*Oreamnos americanus*) consisting of a sire and two offspring, died of pulmonary *M. tuberculosis* at 6 yr of age. The goat's asymptomatic herdmates were screened and had negative chest radiographs and tracheal wash cultures, but one of the two goats was positive on tuberculin skin-test. In October of 1998, a clinically normal Black rhinoceros (*Diceros bicornis*) was diagnosed with *Mycobacterium tuberculosis* after a positive skin test and nasal wash culture. In the winter of 1998, the two remaining goats were evaluated again with negative chest radiographs and tracheal wash cultures. However, 1 yr later, both were humanely euthanized at 8 and 12 yr of age due to clinical evidence of tuberculosis on chest radiographs (both animals), and active clinical signs in one (neither were able to be orally treated). In January of 2001, a rhino was humanely euthanized after a protracted illness that was nonresponsive to aggressive treatment. The rhino was found to have severe multifocal hemosiderosis and atypical mycobacterial infection in her lungs, with no *M. tuberculosis* cultured. This animal had been treated with oral Isoniazid and Rifampin for 1 yr, cultured routinely, and was never culture positive again.

Epidemiologic Investigation

Investigators examined medical and location histories of the affected animals, animal handling practices, health-care procedures, and performed an infection control assessment of the animal compounds and health-care facilities (including measuring air flow in the compounds by smoke testing). We conducted a review of zoo employee medical records for evidence of TB symptoms, tuberculin skin-test results, and chest radiograph

information. A list of current and former employees was cross-matched with reported TB cases in the California state registry from 1985 to 2000. As part of the annual occupational health screening in June of 2000, zoo employees underwent questioning regarding TB symptoms, received tuberculin skin tests, and completed a questionnaire on medical history, job type, and history of contact with the infected animals.

Epidemiologic Findings

No common cross-species contact outside the animal compounds and no contact with an infectious human were found. The distance at which the public was kept from the animals and the distance of the compounds from each other (the elephant compound was 27 meters from the rhino compound and the goat compound was 90 m from both) suggests that direct transmission was unlikely. No active TB cases in humans were found, and no matches were found in the database of reported cases. The RFLP analysis of this strain of *M. tuberculosis* matched that of three elephants with which #1 and #2 were housed at a private elephant facility from September of 1993-February of 1994.¹ We hypothesize that elephants #1 and #2 were infected at the private facility and were shipped with latent *M. tuberculosis* infection in 1994, subsequently infecting the black rhino and Mountain goats at the Los Angeles Zoo.

Of interest, animal caretaking and animal contact were not associated with a positive tuberculin skin-test, while groundskeepers were found to have an increased risk of tuberculin skin-test conversion compared with other job categories. Employees attending the elephant necropsy and employees who trained elephants were more likely to have tuberculin skin-test conversion than those who did not.

Conclusion

This is the first documented human and veterinary epidemiologic investigation of *Mycobacterium tuberculosis* affecting multiple species in a zoo.² No evidence of transmission from humans to animals or active infections in humans were found. Genotyping evidence strongly suggests transmission from one species to another, although no evidence of transmission was discovered. Human tuberculin skin-test conversions associated with the elephants were most likely due to lack of respiratory protection for these employees when the risk of TB infection was not known. The finding that groundskeepers and not animal handlers were associated with a higher risk of tuberculin skin-test conversion was surprising, and we hypothesized that this may have to do with groundskeepers as a group being more likely to have been born outside of the United States.

Control measures to eliminate the spread of disease to people and animals were undertaken immediately and throughout this outbreak, and no further cases of *M. tuberculosis* have been diagnosed at the zoo in the past 3 yr despite ongoing surveillance. Four elephants and three rhinos that had direct contact with the infected animals remain TB negative by trunk and nasal wash culture methods as outlined by the USDA for elephant TB surveillance. Methods of indirect transmission in mammalian zoo species and causes of variability in infection and morbidity within and among species warrant further investigation. Ongoing vigilance, occupational health programs and infection control measures in potentially exposed animals are recommended to prevent ongoing transmission of *M. tuberculosis* in zoo settings.

Acknowledgments

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Recently, human tuberculosis researchers working with tuberculosis in humans have developed an immunoassay that detects a serum protein complex (the antigen 85, or Ag85, complex) produced by mycobacteria in the early stages of mycobacterial infections¹. Previous work has shown that this method is a promising diagnostic tool in the evaluation of tuberculosis exposure in some primate (including orangutan (*Pongo pygmaeus*), a species known for non-specific tuberculin responses)² and captive hoofstock species³. In order to determine the feasibility and applicability of a widespread use of this method for captive and free-ranging wildlife species, we have undertaken a number of pilot studies on different populations of interest, with the goals of optimizing and validating the immunoassay through analysis of serum from known infected and non-infected individuals and through comparisons with other diagnostic methods. Thus far, we have begun evaluating the applicability of the antigen 85 immunoassay in various avian, primate, rhinoceros and hoofstock species for detecting tuberculosis and/or paratuberculosis (Johne's disease) infections. Preliminary results, a summary of which will be presented, indicate that this method may be a valuable adjunct to other testing methods (including gamma interferon and multiple-antigen ELISA) to allow a better evaluation of true mycobacterial status in these species.

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Abstract: From 1997 to 2000, *Mycobacterium tuberculosis* was diagnosed in two Asian elephants (*Elephas maximus*), three Rocky Mountain goats (*Oreamnos americanus*), and one black rhinoceros (*Diceros bicornis*) in the Los Angeles Zoo. DNA fingerprint patterns suggested recent transmission. An investigation found no active cases of tuberculosis in humans; however, tuberculin skin-test conversions in humans were associated with training elephants and attending an elephant necropsy.
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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.
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animals was determined by discriminant analysis. The resulting classification functions were used to calculate the percentage of animals that were correctly classified (i.e., specificity and sensitivity). Of the 77 elephants sampled, 47 fit the criteria for inclusion in discriminant analysis. Of these, seven Asian elephants were considered infected; 25 Asian elephants and 15 African elephants were considered noninfected. The remaining elephants had been exposed to one or more infected animals. The specificity and sensitivity of the multiple-antigen ELISA were both 100% (91.9-100% and 54.4-100%, respectively) with 95% confidence intervals. *M. bovis* culture filtrate showed the highest individual antigen specificity (95%; 83.0-100%) and sensitivity (100%; 54.4-100%). Serum samples from 34 elephants were analyzed over time by the response to the culture filtrate antigen; four of these elephants were culture positive and had been used to calculate the discriminant function. Limitations such as sample size, compromised ability to ascertain each animal's true infection status, and absence of known-infected African elephants suggest that much additional research needs to be conducted regarding the use of this ELISA. However, the results indicate that this multiple-antigen ELISA would be a valuable screening test for detecting *M. tuberculosis* infection in elephant herds.

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Abstract: Antemortem diagnosis of tuberculosis in captive wild animals is often difficult. In addition to the variability of host cellular immune response, which does not always indicate current active infection, reactivity to saprophytic or other mycobacteria is common and may interfere with the interpretation of the intradermal tuberculin skin test. Furthermore, the immobilization required for administering the test and evaluating skin reactions in these animals may result in unacceptable levels of morbidity and mortality, of particular concern in individuals of rare or endangered species. Proteins of the antigen 85 (Ag85) complex are major secretory products of actively metabolizing mycobacteria in vitro. Production of these proteins by mycobacteria during growth in vivo could result in increases in circulating levels of Ag85 in hosts with active tuberculosis. A dot blot immunoassay has been used to detect and quantify circulating Ag85 in captive wild animals with tuberculosis. Elevated levels of Ag85 were observed in animals with active tuberculosis as compared with uninfected animals. Study populations included a herd of nyala (*Tragelaphus angasi*) (n=9) with no history of exposure to *Mycobacterium bovis*. Serum Ag85 levels ranged from <5 to 15 uU/ml (median, 5 uU/ml). The other group included 11 animals from a mixed collection with a documented history of an *M. bovis* outbreak. Animals with pulmonary granulomatous lesions (n=3) had serum Ag85 levels ranging from 320 to 1,280 uU/ml (median, 320 uU/ml). Animals with only chronic mediastinal or mesenteric lymphadenitis (n=4) had serum Ag85 levels ranging from <5 to 80 uU/ml (median, <5 uU/ml). This assay could provide an important adjunct to intradermal skin testing for antemortem diagnosis of tuberculosis in nondomestic species.
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this purpose tuberculins with a well controlled high potency and specificity should be used. In order to diagnose hyperergic or anergic animals it is recommended to use PPD tuberculin with double strength (2 mg tuberculoprotein per ml) or to double the dose (0.2 ml instead of 0.1 ml), so that about 10,000 I.U. are applied. A strict interpretation scheme can increase the efficacy of the test, in particular in the comparative test. In order to improve the diagnosis, we have studied for some years the use of the ELISA which corresponds with humoral immunity.

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cent of warthog were found to show gross lesions on autopsy and of these organisms which could be typed, *Mycobacterium bovis* was isolated in 2 of 6 cases and 5 atypical mycobacterial strains were isolated from the remaining 4. The distribution and character of the lesions is described and it is concluded that the route of infection in the warthog is alimentary. A mycobacterial survey of 8 other species of mammals, 7 species of birds, 5 species of fish and 1 species of amphibian is described. None of the mammals (except possibly 1 elephant), birds, fish or amphibian was harbouring atypical, probably saprophytic, mycobacterial types. The origin of tuberculosis in buffalo and warthog in the Ruwenzori National Park is discussed and is concluded to have been previous contact with domestic cattle.

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