

Elephant Tuberculosis References (By date; most recent first)
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Szydłowski, M. (2022). "Elephants in Nepal: Correlating disease, tourism, and welfare." *Journal of Applied Animal Welfare Science*.

Asian elephants and humans have long shared their lives, but recent changes in human perspectives on animal use have created ripples through the small country of Nepal. Captive elephants are caught in the crossfire between local communities, elephant owners, mahouts, and NGOs in debates over their treatment, health, welfare and use in tourism. In addition, zoonotic disease, natural disasters and political strife affect the lives of captive elephants and mahouts. For example, during the COVID-19 pandemic, elephants, caregivers and owners found themselves facing income loss, decreased welfare from housing and husbandry issues, and food shortages. Many owners sold elephants, fired mahouts, and "quit" the tourism industry. Others sought help from outside organizations, community members, and governmental agencies to retain ownership of what they viewed as valuable commodities. NGOs and grassroots organizations assisted in the hopes of keeping elephants in Nepal, thus preventing them from long, treacherous walks across the border and into situations where they might face further welfare decreases. This article combines elephant stable visits and interviews with mahouts, owners, NGO, and government staff between January 2019 and December 2021. It highlights the ongoing health and welfare challenges faced by elephants and mahouts in Nepal. © 2022 Informa UK Limited, trading as Taylor & Francis Group.

Shah, Y., S. Paudel, K. Pandey, G. P. Gupta, E. S. Solo, J. Joshi, D. K. Pant and B. D. Pandey (2022). "Insights into transmission dynamics of Mycobacterium tuberculosis complex in Nepal." *Tropical Medicine and Health* 50(1).

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis complex (MTBC) in humans and animals. Numbers of multi drug resistance TB (MDR-TB), extrapulmonary TB (EPTB) and zoonotic TB cases are increasingly being reported every year in Nepal posing a major public health problem. Therefore, the Government of Nepal should act immediately to strengthen the screening facilities across the country to be able to identify and treat the TB infected patients as well as detect zoonotic TB in animal species. Endorsement of One Health Act by the Government of Nepal is an opportunity to initiate the joint programs for TB surveillance among human and animal species using one health approach to reduce the TB burden in Nepal. © 2022, The Author(s).

Ishikawa, S., Y. Ozeki, S. Suga, Y. Mukai, H. Kobayashi, E. Inouchi, S. A. Kaboso, G. Gebretsadik, D. Dewi, A. Nishiyama, Y. Tateishi, H. Takihara, S. Okuda, S. Yoshida, N. Misawa and S. Matsumoto (2022). "Monitoring IgG against Mycobacterium tuberculosis " *Sci Rep* 12(1): 4310.

Tuberculosis (TB) is fatal in elephants, hence protecting elephants from TB is key not only in the conservation of this endangered animal, but also to prevent TB transmission from elephants to humans. Most human TB cases arise from long-term asymptomatic infections. Significant diagnostic challenges remain in the detection of both infection and disease development from latency in elephants due to their huge bodies. In this study, we assessed cryopreserved sera collected for over

16 years, from the first Japanese treatment case of elephant TB. Semi-quantification of IgG levels to 11 proteins showed high detection levels of 3 proteins, namely ESAT6/CFP10, MPB83 and Ag85B. The level of IgG specific to these 3 antigens was measured longitudinally, revealing high and stable ESAT6/CFP10 IgG levels regardless of onset or treatment. Ag85B-specific IgG levels were largely responsive to onset or treatment, while those of MPB83 showed intermediate responses. These results suggest that ESAT6/CFP10 is immunodominant in both asymptomatic and symptomatic phases, making it useful in the detection of infection. On the other hand, Ag85B has the potential to be a marker for the prediction of disease onset and in the evaluation of treatment effectiveness in elephants.

Goosen, W. J., L. Kleynhans, T. J. Kerr, P. D. van Helden, P. Buss, R. M. Warren and M. A. Miller (2022). "Improved detection of *Mycobacterium tuberculosis* and *M. bovis* in African wildlife samples using cationic peptide decontamination and mycobacterial culture supplementation." *J Vet Diagn Invest* 34(1): 61-67.

In South Africa, mycobacterial culture is regarded as the gold standard for the detection of *Mycobacterium tuberculosis* complex (MTBC) infection in wildlife even though it is regarded as "imperfect." We compared a novel decontamination and mycobacterial culture technique (TiKa) to the conventional mycobacterium growth indicator tube (MGIT) system using known amounts of bacilli and clinical samples from MTBC-infected African buffaloes (*Syncerus caffer*), white rhinoceros (*Ceratotherium simum*), and African elephants (*Loxodonta africana*). Use of the TiKa-KiC decontamination agent on samples spiked with 10,000 to 10 colony forming units (cfu) of *M. bovis* (SB0121) and *M. tuberculosis* (H37Rv) had no effect on isolate recovery in culture. In contrast, decontamination with MGIT MycoPrep resulted in no growth of *M. bovis* samples at concentrations < 1,000 cfu and *M. tuberculosis* samples < 100 cfu. Subsequently, we used the TiKa system with stored clinical samples (various lymphatic tissues) collected from wildlife and paucibacillary bronchoalveolar lavage fluid, trunk washes, and endotracheal tube washes from 3 species with known MTBC infections. Overall, MTBC recovery by culture was improved significantly ($p < 0.01$) by using TiKa compared to conventional MGIT, with 54 of 57 positive specimens versus 25 of 57 positive specimens, respectively. The TiKa mycobacterial growth system appears to significantly enhance the recovery of MTBC members from tissue and paucibacillary respiratory samples collected from African buffaloes, African elephants, and white rhinoceros. Moreover, the TiKa system may improve success of MTBC culture from various sample types previously deemed unculturable from other species.

Tollis, M., E. Ferris, M. S. Campbell, V. K. Harris, S. M. Rupp, T. M. Harrison, W. K. Kiso, D. L. Schmitt, M. M. Garner, C. A. Aktipis, C. C. Maley, A. M. Boddy, M. Yandell, C. Gregg, J. D. Schiffman and L. M. Abegglen (2021). "Elephant Genomes Reveal Accelerated Evolution in Mechanisms Underlying Disease Defenses." *Mol Biol Evol* 38(9): 3606-3620.

Disease susceptibility and resistance are important factors for the conservation of endangered species, including elephants. We analyzed pathology data from 26 zoos and report that Asian elephants have increased neoplasia and malignancy prevalence compared with African bush elephants. This is consistent with observed higher susceptibility to tuberculosis and elephant endotheliotropic herpesvirus (EEHV) in Asian elephants. To investigate genetic mechanisms underlying disease resistance, including differential responses between species, among other elephant traits, we sequenced multiple elephant genomes. We report a draft assembly for an Asian elephant, and defined 862 and 1,017 conserved potential regulatory elements in Asian and African bush elephants, respectively. In the genomes of both elephant species, conserved elements were significantly enriched with genes differentially expressed between the species. In Asian elephants, these putative regulatory regions were involved in immunity pathways including tumor-necrosis factor, which plays an important

role in EEHV response. Genomic sequences of African bush, forest, and Asian elephant genomes revealed extensive sequence conservation at TP53 retrogene loci across three species, which may be related to TP53 functionality in elephant cancer resistance. Positive selection scans revealed outlier genes related to additional elephant traits. Our study suggests that gene regulation plays an important role in the differential inflammatory response of Asian and African elephants, leading to increased infectious disease and cancer susceptibility in Asian elephants. These genomic discoveries can inform future functional and translational studies aimed at identifying effective treatment approaches for ill elephants, which may improve conservation.

Suga, S., Y. Mukai, S. Ishikawa, S. Yoshida, S. Paudel and T. Wada (2021). "Intensive treatment of a captive bornean elephant (*Elephas maximus borneensis*) infected with *Mycobacterium caprae* in Japan." *Journal of Zoo and Wildlife Medicine* 51(4): 1062-1066.

In 2015, an estimated 17-year-old female Bornean elephant (*Elephas maximus borneensis*) at Fukuyama Zoo in Japan exhibited anorexia and significant weight loss. Pan-susceptible *Mycobacterium tuberculosis* complex (MTBC) was isolated from vaginal discharge, oral mucus, urine, and fecal samples by culture. The isolate was identified as *Mycobacterium caprae* by genetic analysis. Isoniazid, pyrazinamide, and levofloxacin were administered rectally. Body weight increased to normal, but subsequently decreased again. Elevation of liver enzymes occurred, likely related to the increase in isoniazid dosage. After recovery from side effects, the elephant's weight increased further. However, isoniazid-resistant *M. caprae* was isolated from oral mucus after anti-tuberculosis drug treatment for 9 mo. The regimen was changed to rifampicin, pyrazinamide, ethambutol, and levofloxacin, administered orally or rectally. The 18-mo treatment was completed in October 2018. This elephant has shown no clinical sign since. No MTBC-positive sample had been obtained as of March 2020. © Copyright 2020 by American Association of Zoo Veterinarians.

Shah, Y. and S. Paudel (2021). "Protect elephants from tuberculosis." *Science* 374(6569): 832-833.

Sahoo, N., S. K. Sahu, A. K. Das, D. Mohapatra, S. K. Panda, S. K. Gupta, B. K. Behera, A. Pahari and M. Dash (2021). "ELEPHANT ENDOTHELIO TROPIC HERPESVIRUS HEMORRHAGIC DISEASE OUTBREAK IN AN INDIAN ZOO." *J Zoo Wildl Med* 52(4): 1286-1297.

Elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV HD) is an acute viral infection of growing Asian elephants (*Elephas maximus*). Four apparently healthy subadult Asian elephants aged between 6 and 10 yr at Nandankanan Zoological Park (NKZP), India, died of EEHV HD during August-September 2019. All four elephants were rescued from different reserved forests of Odisha state at less than 1 yr of age and hand reared in the NKZP. Elephants exhibited the clinical signs of lethargy, head swelling, fever, loss of appetite, abdominal distension, scant urination and defecation, signs of colic, lameness, trunk discharge, cyanosis/ulceration of tongue, erratic behavior, and recumbence before death. Period of illness varied between 28 and 42 h. Thrombocytopenia was the common significant hematological observation. No significant biochemical alterations were recorded except for higher creatinine concentrations. Analysis of blood samples in RT-PCR assay using two different sets of primers and probes that targeted terminase gene and major DNA-binding protein gene followed by cPCR and sequencing was positive for EEHV-1A in all four animals. Postmortem examination of all four carcasses showed hemorrhages in internal organs, including the hard palate, heart, lungs, stomach, mesenteric lymph nodes, mesentery, colon serosa, spleen, liver, kidney, and meninges. Histopathology showed congestion and/or hemorrhages in heart, lung, brain, kidney, and liver. There was presence of intranuclear inclusion bodies in the sinusoidal epithelial cells. The outbreak of EEHV HD that resulted in the acute death of four juvenile captive Asian elephants within <30 d, the first of its kind documented in India, is increasing the fear of similar outbreaks in the future.

Rajhans, U., G. Wankhede, B. Ambore, S. Chaudhari, N. Nighot, V. Dhaygude and C. Sonekar (2021). "Sero-diagnosis of Tuberculosis in Elephants in Maharashtra, India." *Journal of Threatened Taxa* 13(7): 18713-18718.

Tuberculosis is a highly contagious zoonotic disease caused by *Mycobacterium* spp. A study was conducted to detect the presence of *Mycobacterium* in captive elephants. A total of 15 captive elephants were screened from various regions in Maharashtra. The blood and serum samples collected were subjected to rapid test kit, BacT/ALERT 3D system, Ziehl-Neelsen (ZN) staining and PCR. All the samples were found seronegative using rapid test kit and whole blood PCR. Whereas, all samples were signalled culture positive in BacT/ALERT 3D system which were further subjected to PCR, only one amplicon was produced of 176bp of RD4 gene (*Mycobacterium bovis*) and no acid-fast organism was detected upon ZN. Due to the atypical nature of this organism, diagnosis of this disease in elephants using various tests is complicated unlike the diagnostic tests that are validated in domestic animals. Therefore, many tests have sub-optimal sensitivity and specificity in elephants. As TB is a zoonotic disease, transmission can occur between human-livestock-elephants interface. Therefore, the zoos and state forest authority should inculcate a protocol of periodic TB screening for Mahouts and elephants in captivity along with protocol of elephant-visitor interaction, thus helping in conservation of this endangered species in India. © Rajhans et al. 2021. Creative Commons Attribution 4.0 International License. JoTT allows unrestricted use, reproduction, and distribution of this article in any medium by providing adequate credit to the author(s) and the source of publication.

Paudel, S., E. P. Brenner, S. A. Hadi, Y. Suzuki, C. Nakajima, T. Tsubota, K. P. Gairhe, B. Maharjan and S. Sreevatsan (2021). "Genome Sequences of Two *Mycobacterium tuberculosis* Isolates from Asian Elephants in Nepal." *Microbiol Resour Announc* 10(36): e0061421.

This report describes the genome sequences of two *Mycobacterium tuberculosis* isolates, S1 and S3, recovered from Asian elephants in Nepal. These genome sequences will enhance our understanding of the genomic epidemiology of *Mycobacterium tuberculosis* in Asian elephants.

Miller, M. A., T. J. Kerr, C. R. de Waal, W. J. Goosen, E. M. Streicher, G. Hausler, L. Rossouw, T. Manamela, L. van Schalkwyk, L. Kleynhans, R. Warren, P. van Helden and P. E. Buss (2021). "Mycobacterium bovis Infection in Free-Ranging African Elephants." *Emerg Infect Dis* 27(3): 990-992.

Mycobacterium bovis infection in wildlife species occurs worldwide. However, few cases of *M. bovis* infection in captive elephants have been reported. We describe 2 incidental cases of bovine tuberculosis in free-ranging African elephants (*Loxodonta africana*) from a tuberculosis-endemic national park in South Africa and the epidemiologic implications of these infections.

Lekko, Y. M., A. Che-Amat, P. T. Ooi, S. Omar, D. T. Mohd-Hamdan, L. S. Linazah, Z. Zakaria, S. Z. Ramanoon, M. Mazlan, F. F. A. Jesse, M. F. A. Abdul-Razak, S. Jasni and N. Abdul-Hamid (2021). "Detection of *Mycobacterium tuberculosis* complex antibodies in free-ranged wild boar and wild macaques in selected districts in Selangor and reevaluation of tuberculosis serodetection in captive Asian elephants in Pahang, Peninsular Malaysia." *J Vet Med Sci* 83(11): 1702-1707.

Tuberculosis (TB) is a chronic inflammatory and zoonotic disease caused by *Mycobacterium tuberculosis* complex (MTBC) members, affecting several domestic animals, wildlife species and humans. The preliminary investigation was aimed to detect antibody against MTBC among indigenous wildlife which are free-ranged wild boar, free-ranged wild macaques and captive Asian elephants in selected areas of Selangor and elephant conservation centre in Pahang, respectively. The results indicate that MTBC serodetection rate in wild boar was 16.7% (7.3-33.5 at 95% confidence interval (CI)) using an in-house ELISA bPPD IgG and 10% (3.5-25.6 at 95% CI) by DPP[®]VetTB assay, while the wild

macaques and Asian elephant were seronegative. The univariate analysis indicates no statistically significant difference in risk factors for sex and age of wild boar but there was a significant positive correlation ($P < 0.05$) between bovine TB in dairy cattle and wild boar seropositivity in the Sepang district.

Kock, R., A. L. Michel, D. Yeboah-Manu, E. I. Azhar, J. B. Torrelles, S. I. Cadmus, L. Brunton, J. M. Chakaya, B. Marais, L. Mboera, Z. Rahim, N. Haider and A. Zumla (2021). "Zoonotic Tuberculosis – The Changing Landscape." *International Journal of Infectious Diseases* 113: S68-S72.

Despite slow reductions in the annual burden of active human tuberculosis (TB) cases, zoonotic TB (zTB) remains a poorly monitored and an important unaddressed global problem. There is a higher incidence in some regions and countries, especially where close association exists between growing numbers of cattle (the major source of *Mycobacterium bovis*) and people, many suffering from poverty, and where dairy products are consumed unpasteurised. More attention needs to be focused on possible increased zTB incidence resulting from growth in dairy production globally and increased demand in low income countries in particular. Evidence of new zoonotic mycobacterial strains in South Asia and Africa (e.g. *M. orygis*), warrants urgent assessment of prevalence, potential drivers and risk in order to develop appropriate interventions. Control of *M. bovis* infection in cattle through detect and cull policies remain the mainstay of reducing zTB risk, whilst in certain circumstances animal vaccination is proving beneficial. New point of care diagnostics will help to detect animal infections and human cases. Given the high burden of human tuberculosis (caused by *M. tuberculosis*) in endemic areas, animals are affected by reverse zoonosis, including multi-drug resistant strains. This, may create drug resistant reservoirs of infection in animals. Like COVID-19, zTB is evolving in an ever-changing global landscape. © 2021 The Author(s)

Jia, P., S. Dai, T. Wu and S. Yang (2021). "New Approaches to Anticipate the Risk of Reverse Zoonosis." *Trends in Ecology and Evolution* 36(7): 580-590.

The coronavirus disease 2019 (COVID-19) pandemic can cause reverse zoonoses (i.e., human–animal transmission of COVID-19). It is vital to utilize up-to-date methods to improve the control, management, and prevention of reverse zoonoses. Awareness of reverse zoonoses should be raised at both individual and regional/national levels for better protection of both humans and animals. © 2021 Elsevier Ltd

Chaney, S. B., D. McAloose, R. Greenwald, K. P. Lyashchenko and P. P. Calle (2021). "ASSESSMENT OF MULTIANTIGEN PRINT IMMUNOASSAY AND RAPID LATERAL-FLOW TEST FOR THE DETECTION OF MYCOBACTERIUM BOVIS INFECTION IN MALAYAN TAPIR (*TAPIRUS INDICUS*)." *J Zoo Wildl Med* 52(4): 1257-1262.

A multiantigen print immunoassay (MAPIA) and rapid test (RT) developed and validated for detection of mycobacterial antibodies in elephants (*Elephas maximus* and *Loxodonta africana*) was assessed in Malayan tapir (*Tapirus indicus*). Retrospective analysis of banked serum from one *Mycobacterium bovis* infected and seven presumably uninfected tapir was performed by MAPIA and RT. A sample collected 2 mon prior to the death of a culture-confirmed *M. bovis*-infected tapir served as a positive control. Seroreactivity of this sample was demonstrated via both MAPIA and RT testing. Seven uninfected animals, including four without postmortem evidence of mycobacterial disease and three that remain healthy, were negative controls; none demonstrated seroreactivity to key antigens with either test. These results suggest that MAPIA and RT have potential utility for rapid detection of *M. bovis* infection in Malayan tapir.

Brenner, E. P., S. A. Hadi, B. Harris, S. Robbe-Austerman and S. Sreevatsan (2021). "Genome Sequences of Mycobacterium Strains Recovered from Captive Elephants with Tuberculosis." *Microbiol Resour Announc* 10(36): e0067121.

Members of the Mycobacterium tuberculosis complex cause tuberculosis, infamous for enormous impacts on human health. As zoonoses, they also threaten endangered species like African/Asian elephants. We report the whole-genome sequences of Mycobacterium tuberculosis bv. tuberculosis and Mycobacterium tuberculosis bv. bovis from two zoo elephants in the United States.

Verma, R., B. M. C. Swift, W. Handley-Hartill, J. K. Lee, G. Woltmann, C. E. D. Rees and P. Haldar (2020). "A novel, high-sensitivity, bacteriophage-based assay identifies low-level mycobacterium tuberculosis bacteremia in immunocompetent patients with active and incipient tuberculosis." *Clinical Infectious Diseases* 70(5): 933-936.

The haematogenous dissemination of Mycobacterium tuberculosis (Mtb) is critical to the pathogenesis of progressive tuberculous infections in animal models. Using a novel, phage-based blood assay, we report the first concordant evidence in well-characterized, immunocompetent human cohorts, demonstrating associations of Mtb bacteremia with progressive phenotypes of latent infection and active pulmonary tuberculosis. © The Author(s) 2019.

Unuma, K., R. Watanabe, N. Hirayama and K. Uemura (2020). "Autopsy Identification of Viable Mycobacterium Tuberculosis in the Lungs of a Markedly Decomposed Body." *Journal of Forensic Sciences* 65(6): 2194-2197.

Various infectious diseases, including COVID-19, MERS, and tuberculosis, are global public health issues. Tuberculosis, which is caused by Mycobacterium tuberculosis (MTB), is highly contagious and can be transmitted through inhalation of the bacteria. However, it has been assumed that the infectiousness of bacteria and viruses in dead bodies weakens as the time from death increases. In particular, there is little awareness of infection control measures concerning decomposed bodies or even the need for such measures. The deceased, in whom we discovered MTB 3 months following her death, was a woman in her 80s who died at home. We performed judicial autopsy, because police suspected homicide when her husband hanged himself. Obtained organs were used for microscopic examination by hematoxylin–eosin staining and Ziehl–Neelsen staining. In addition, real-time PCR and mycobacterial culture testing using Ogawa's medium were performed for the detection of MTB. We found that the MTB in the decomposed body remained viable and potentially infectious. To identify the bacterial strain further, we performed DNA-DNA hybridization and identified the strain as MTB complex. Potentially infectious live MTB survived in the dead body far longer than had been previously reported. Pathologists should consider microbial culture tests for all autopsied cases in which the decedent's medical history or macro-examination suggests possible infection, even when a long duration of time has passed since death. Pathologists and specialists who perform autopsies should recognize that all dead bodies are potentially infectious, including those in which long periods have elapsed since death. © 2020 American Academy of Forensic Sciences

Swift, B. M. C., N. Meade, E. S. Barron, M. Bennett, T. Perehenic, V. Hughes, K. Stevenson and C. E. D. Rees (2020). "The development and use of Actiphage® to detect viable mycobacteria from bovine tuberculosis and Johne's disease-infected animals." *Microbial Biotechnology* 13(3): 738-746.

Here, we describe the development of a method that exploits bacteriophage D29 as a lysis agent for efficient DNA extraction from low numbers of mycobacterial cells. This method (Actiphage®) used in combination with PCR achieved rapid and sensitive ($LOD \leq 10 \text{ cell ml}^{-1}$) detection and identification of viable, pathogenic mycobacteria in blood samples within 6 h. We demonstrate that mycobacteriophage D29 can be used to detect a range of mycobacteria from clinical blood samples

including both *Mycobacterium tuberculosis* complex and *Mycobacterium avium* subsp. paratuberculosis without the need for culture and confirms our earlier observations that a low-level bacteraemia is associated with these infections in cattle. In a study of *M. bovis*-infected cattle (n = 41), the sensitivity of the Actiphage® method was 95 % (95 % CI; 0.84–0.99) and specificity was 100 % (95% CI; 0.92–1). We further used Actiphage® to demonstrate viable *Mycobacterium avium* subsp. paratuberculosis is present in the blood of Johne's infected cattle. This method provides a revolutionary new tool for the study of infections caused by these difficult to grow pathogens. © 2019 The Authors. Microbial Biotechnology published by John Wiley & Sons Ltd and Society for Applied Microbiology.

Sookaromdee, P. and V. Wiwanitkit (2020). "Zoonotic possibility of tuberculosis from domestic elephants: a case assessment from Thailand." *Egyptian Journal of Chest Diseases and Tuberculosis* 69(3): 447-448.

Background Tuberculosis is an important medical problem which is at present a public health problem around the world. Zoonotic tuberculosis is a new emerging problem and has become an important issue today. The elephant tuberculosis is the specific kind of animal tuberculosis. Zoonotic tuberculosis from elephants is an interesting situation that becomes the new concern in the community where domestic elephants are common. Methods In this article, the authors specifically perform a mathematical model study to assess zoonotic possibility of tuberculosis from domestic elephants based on the available data in Thailand. Results According to this study, the prediction on the transmission rate is equal to 54.5% focusing on zoonotic transmission from domestic elephants to humans. Conclusion In this article, the authors assessed the possibility of zoonotic tuberculosis from the domestic elephant. It can be seen that there is a high chance.

Songthammanuphap, S., S. Puthong, C. Pongma, A. Buakeaw, T. Prammananan, S. Warit, W. Tipkantha, E. Kaewkhunjob, W. Yindeeyoungyeon and T. Palaga (2020). "Detection of *Mycobacterium tuberculosis* complex infection in Asian elephants (*Elephas maximus*) using an interferon gamma release assay in a captive elephant herd." *Sci Rep* 10(1): 14551.

Tuberculosis is highly contagious disease that can be transmitted between humans and animals. Asian elephants (*Elephas maximus*) in captivity live in close contact with humans in many Asian countries. In this study, we developed an interferon gamma release assay (IGRA) for elephant TB detection using antigens from the MTB complex (MTBC) and nontuberculous mycobacteria (NTM) as stimulating antigens (PPD, ESAT6, CFP10) to elicit a cell-mediated immune response (CMIR). The developed assay was applied to an elephant herd of more than 60 animals in Thailand, and the results were compared with those obtained through serological detection. IGRA has sufficient sensitivity for detecting elephant interferon gamma (eIFN γ) from specific antigen-stimulated PBMCs. Among 60 animals tested, 20 samples (33.3%) showed negative results for both MTBC and NTM infection. Eighteen samples (30%) showed positive responses against PPD from *M. bovis* and/or ESAT6 and CFP10, indicating MTBC infection. In contrast, only 15.6% showed seropositivity in a commercial serological test kit for elephant TB. The discrepancies between serological and CMIR highlight that the two methods may detect different stages of elephant TB. Therefore, employing both tests may enable them to complement each other in correctly identifying elephants that have been exposed to MTBC.

Ruetten, M., H. W. Steinmetz, M. Thiersch, M. Kik, L. Vaughan, S. Altamura, M. U. Muckenthaler and M. Gassmann (2020). "Iron Regulation in Elderly Asian Elephants (*Elephas maximus*) Chronically Infected With *Mycobacterium tuberculosis*." *Front Vet Sci* 7: 596379.

Restriction of nutrients to pathogens (nutritional immunity) is a critical innate immune response mechanism that operates when pathogens such as *Mycobacterium tuberculosis* have the

potential to evade humoral immunity. Tuberculosis is of growing concern for zoological collections worldwide and is well-illustrated by infections of Asian and African elephants, where tuberculosis is difficult to diagnose. Here, we investigated hematological parameters and iron deposition in liver, lung, and spleen of three Asian elephants (*Elephas maximus*) infected with *Mycobacterium tuberculosis*. For reference purposes, we analyzed tissue samples from control *M. tuberculosis*-negative elephants with and without evidence of inflammation and/or chronic disease. Molecular analyses of bacterial lesions of post mortally collected tissues confirmed *M. tuberculosis* infection in three elephants. DNA sequencing of the bacterial cultures demonstrated a single source of infection, most likely of human origin. In these elephants, we observed moderate microcytic anemia as well as liver (mild), lung (moderate) and spleen (severe) iron accumulation, the latter mainly occurring in macrophages. Macrophage iron sequestration in response to infection and inflammation is caused by inhibition of iron export via hepcidin-dependent and independent mechanisms. The hepatic mRNA levels of the iron-regulating hormone hepcidin were increased in only one control elephant suffering from chronic inflammation without mycobacterial infection. By contrast, all three tuberculosis-infected elephants showed low hepcidin mRNA levels in the liver and low serum hepcidin concentrations. In addition, hepatic ferroportin mRNA expression was high. This suggests that the hepcidin/ferroportin regulatory system aims to counteract iron restriction in splenic macrophages in *M. tuberculosis* infected elephants to provide iron for erythropoiesis and to limit iron availability for a pathogen that predominantly proliferates in macrophages. Tuberculosis infections appear to have lingered for more than 30 years in the three infected elephants, and decreased iron availability for mycobacterial proliferation may have forced the bacteria into a persistent, non-proliferative state. As a result, therapeutic iron substitution may not have been beneficial in these elephants, as this therapy may have enhanced progression of the infection.

Peters, H., A. Sadaula, N. Masters and A. Sainsbury (2020). "Risks from disease caused by *Mycobacterium orygis* as a consequence of Greater one-horned Rhinoceros (*Rhinoceros unicornis*) translocation in Nepal." *Transboundary and Emerging Diseases* 67(2): 711-723.

The greater one-horned rhinoceros (*Rhinoceros unicornis*) is listed as vulnerable by the IUCN Red List. *Mycobacterium orygis*-associated disease was identified in a single greater one-horned rhino in Chitwan National Park in February 2015 prior to a planned translocation of five greater one-horned rhinoceros from Chitwan National Park to Bardia National Park for conservation purposes. This paper describes a qualitative disease risk analysis conducted retrospectively post-translocation for *Mycobacterium orygis* and this translocation, with the aim to improve the understanding of disease threats to the conservation of greater one-horned rhino. The disease risk analysis method used was devised by Sainsbury & Vaughan-Higgins (*Conservation Biology*, 26, 2017, 442) with modifications by Bobadilla Suarez et al (*EcoHealth*, 14, 2017, 1) and Rideout et al (*EcoHealth*, 14, 2017, 42) and included the use of a scenario tree and an analysis of uncertainty as recommended by Murray et al. (*Handbook on import risk analysis for animals and animal products. Volume 1. Introduction and qualitative risk analysis*, 2004), and the first time this combination of methods has been used to assess the risk from disease in a conservation translocation. The scenario tree and analysis of uncertainty increased the clarity and transparency of the analysis. Rideout et al.'s (*EcoHealth*, 14, 2017, 42) criteria were used to assess the source hazard and may be useful in comparative assessment of source hazards for future conservation translocations. The likelihood of release into the destination site of *Mycobacterium orygis* as a source hazard was estimated as of low risk, the risk of exposure of populations at the destination was of high risk and the likelihood of biological and environmental consequences was low. Overall, the risk from disease associated with *Mycobacterium orygis* as a result of this translocation was found to be low. Recommendations on disease risk management strategies could be improved with a better

understanding of the epidemiology including the presence/absence of *Mycobacterium orygis* in greater one-horned rhino to develop effective disease risk management strategies.

Paudel, S. and S. Sreevatsan (2020). "Tuberculosis in elephants: Origins and evidence of interspecies transmission." *Tuberculosis* 123.

Tuberculosis (TB) is a devastating disease in elephants caused by either *Mycobacterium tuberculosis* or *M. bovis*. It is an ancient disease, and TB in elephants was first reported over two millennia ago in Sri Lanka. Outbreaks of TB worldwide, in captive and free-ranging elephant populations, have been recorded. Interspecies transmission of TB among elephants and humans has been confirmed in several geographic localities using spoligotyping, MIRU-VNTR analysis, and/or comparative genomics. Active surveillance of TB in wild and captive elephants and their handlers is necessary to prevent TB transmission at the elephant-human interface and to aid in the conservation of Asian and African elephants. In this review, we present an overview of diagnosis, reports of TB outbreaks in the past 25 years, TB in wild elephants, its transmission, and possible prevention and control strategies that can be applied at the elephant-human interface. © 2020

Motlatso, H. T. and R. M. Mogano (2020). "Utility of xpert® MTB/RIF ultra assay in the rapid diagnosis of bovine T tuberculosis in wildlife and livestock animals from South Africa." *Prev Vet Med* 177.

Goosen, W. J., T. J. Kerr, L. Kleynhans, R. M. Warren, P. D. van Helden, D. H. Persing, S. D. C. Parsons, P. Buss and M. A. Miller (2020). "The Xpert MTB/RIF Ultra assay detects *Mycobacterium tuberculosis* complex DNA in white rhinoceros (*Ceratotherium simum*) and African elephants (*Loxodonta africana*)." *Sci Rep* 10(1): 14482.

The study describes the novel use of the Xpert MTB/RIF Ultra assay for detection of *Mycobacterium tuberculosis* complex (MTBC) DNA in samples from white rhinoceros (*Ceratotherium simum*) and African elephants (*Loxodonta africana*). Culture negative respiratory sample matrices were spiked to determine if the Ultra could detect MTBC DNA in rhinoceros and elephant samples. Rhinoceros bronchial alveolar lavage fluid (BALF) was found to have an inhibitory effect on the Ultra. In this study, the limit of detection (LOD) of *M. tuberculosis* H37Rv in all spiked animal samples were 2 CFU/ml compared to 15.6 CFU/ml for humans, while the LOD for *M. bovis* SB0121 was 30 CFU/ml compared to 143.4 CFU/ml for *M. bovis* BCG in humans. Screening was performed on stored tissue and respiratory samples from known MTBC-infected animals and MTBC DNA was detected in 92% of samples collected from six rhinoceros and two elephants. Conversely, 83% of culture-negative tissue and respiratory samples from uninfected animals tested negative on the Ultra. In conclusion, the Ultra assay appears to be a sensitive and rapid diagnostic test for the detection of MTBC DNA from tissue and respiratory samples collected from African elephants and rhinoceros. Furthermore, the Ultra assay could provide a new tool for the detection of MTBC in various sample types from other wildlife species.

Goosen, W. J., T. J. Kerr, L. Kleynhans, P. Buss, D. Cooper, R. M. Warren, P. D. van Helden, B. Schröder, S. D. C. Parsons and M. A. Miller (2020). "The VetMAX™ *M. tuberculosis* complex PCR kit detects MTBC DNA in antemortem and postmortem samples from white rhinoceros (*Ceratotherium simum*), African elephants (*Loxodonta africana*) and African buffaloes (*Syncerus caffer*)." *BMC Vet Res* 16(1): 220.

BACKGROUND: Bovine tuberculosis and tuberculosis are chronic infectious diseases caused by the *Mycobacterium tuberculosis* complex members, *Mycobacterium bovis* and *Mycobacterium tuberculosis*, respectively. Infection with *M. bovis* and *M. tuberculosis* have significant implications for wildlife species management, public health, veterinary disease control, and conservation endeavours. **RESULTS:** Here we describe the first use of the VetMAX™ *Mycobacterium tuberculosis* complex (MTBC) DNA quantitative real-time polymerase chain reaction (qPCR) detection kit for African wildlife samples.

DNA was extracted from tissues harvested from 48 African buffaloes and MTBC DNA was detected (test-positive) in all 26 *M. bovis* culture-confirmed animals with an additional 12 PCR-positive results in culture-negative buffaloes (originating from an exposed population). Of six MTBC-infected African rhinoceros tested, MTBC DNA was detected in antemortem and postmortem samples from five animals. The PCR was also able to detect MTBC DNA in samples from two African elephants confirmed to have *M. bovis* and *M. tuberculosis* infections (one each). Culture-confirmed uninfected rhinoceros and elephants' samples tested negative in the PCR assay. CONCLUSIONS: These results suggest this new detection kit is a sensitive screening test for the detection of MTBC-infected African buffaloes, African elephants and white rhinoceros.

Budvytiene, I. and N. Banaei (2020). "Simple processing of formalin-fixed paraffin-embedded tissue for accurate testing with the xpert MTB/RIF assay." *Journal of Clinical Microbiology* 58(3).

Rosen, L. E., F. Olea-Popelka, S. L. Deem, R. Isaza, D. Schmitt and M. Miller (2019). "SURVEY OF ANTITUBERCULOSIS DRUG ADMINISTRATION AND ADVERSE EFFECTS IN ELEPHANTS IN NORTH AMERICA." *J Zoo Wildl Med* 50(1): 23-32.

Tuberculosis, caused by *Mycobacterium tuberculosis*, is a disease causing morbidity and mortality in captive elephants (*Elephas maximus* and *Loxodonta africana*) as well as free-ranging individuals. Elephants in North America diagnosed with tuberculosis are often treated with antituberculosis drugs, unlike livestock species, which has necessitated the development of treatment guidelines adapted from recommendations for humans. There are few published reports describing empirical treatment, which may be complicated by poor patient compliance, interruptions in drug administration, and adverse effects. A survey of elephants in North America was conducted to compile information on treatment protocols, including drugs, dosages, routes of administration, serum drug concentrations, and adverse effects of antituberculosis treatment. Responses were received regarding 182 elephants, 12 of which were treated prophylactically or therapeutically with antituberculosis drugs. Treatment protocols varied among elephants, and included various combinations of isoniazid, rifampin, pyrazinamide, ethambutol, enrofloxacin, levofloxacin, and ethionamide. Serum drug concentrations also varied considerably among and within individuals. Facility staff reported 5 elephants (out of 7 treated elephants with responses) that exhibited clinical signs that may have been associated with antituberculosis drugs or treatment procedures. Anorexia, decreased water intake, constipation, depression, ataxia, limb paresis, and tremors were among the signs observed. Most adverse effects were reported to be moderate or severe, resulting in interruption of the treatment. The results from this survey provide veterinarians and elephant managers with valuable historical data to make informed clinical management decisions regarding antituberculosis therapy in elephants.

Paudel, S., T. Tsubota and S. K. Mikota (2019). "Human TB threat to wild elephants." *Nature* 571(7764): 174.

Paudel, S., C. Nakajima, S. K. Mikota, K. P. Gairhe, B. Maharjan, S. Subedi, A. Poudel, M. Sashika, M. Shimozuru, Y. Suzuki and T. Tsubota (2019). "Mixed *Mycobacterium tuberculosis* Lineage Infection in 2 Elephants, Nepal." *Emerg Infect Dis* 25(5): 1031-1032.

Tuberculosis in elephants is primarily caused by *Mycobacterium tuberculosis*. We identified mixed *M. tuberculosis* lineage infection in 2 captive elephants in Nepal by using spoligotyping and large sequence polymorphism. One elephant was infected with Indo-Oceanic and East African-Indian (CAS-Delhi) lineages; the other was infected with Indo-Oceanic and East Asian (Beijing) lineages.

Paudel, S., S. K. Mikota and T. Tsubota (2019). "Tuberculosis threat in Asian elephants." *Science* 363(6425): 356.

Miller, M. A., P. Buss, E. O. Roos, G. Hausler, A. Dippenaar, E. Mitchell, L. van Schalkwyk, S. Robbe-Austerman, W. R. Waters, A. Sikar-Gang, K. P. Lyashchenko, S. D. C. Parsons, R. Warren and P. van Helden (2019). "Fatal Tuberculosis in a Free-Ranging African Elephant and One Health Implications of Human Pathogens in Wildlife." *Front Vet Sci* 6: 18.

Tuberculosis (TB) in humans is a global public health concern and the discovery of animal cases of *Mycobacterium tuberculosis* (Mtb) infection and disease, especially in multi-host settings, also has significant implications for public health, veterinary disease control, and conservation endeavors. This paper describes a fatal case of Mtb disease in a free-ranging African elephant (*Loxodonta africana*) in a high human TB burden region. Necropsy revealed extensive granulomatous pneumonia, from which Mtb was isolated and identified as a member of LAM3/F11 lineage; a common lineage found in humans in South Africa. These findings are contextualized within a framework of emerging Mtb disease in wildlife globally and highlights the importance of the One Health paradigm in addressing this anthroponotic threat to wildlife and the zoonotic implications.

Martinez, L., R. Verma, J. Croda, C. R. Horsburgh, Jr., K. S. Walter, N. Degner, K. Middelkoop, A. Koch, S. Hermans, D. F. Warner, R. Wood, F. Cobelens and J. R. Andrews (2019). "Detection, survival and infectious potential of *Mycobacterium tuberculosis* in the environment: a review of the evidence and epidemiological implications." *The European respiratory journal* 53(6).

Much remains unknown about *Mycobacterium tuberculosis* transmission. Seminal experimental studies from the 1950s demonstrated that airborne expulsion of droplet nuclei from an infectious tuberculosis (TB) patient is the primary route of transmission. However, these findings did not rule out other routes of *M. tuberculosis* transmission. We reviewed historical scientific evidence from the late 19th/early 20th century and contemporary studies investigating the presence, persistence and infectiousness of environmental *M. tuberculosis*. We found both experimental and epidemiological evidence supporting the presence and viability of *M. tuberculosis* in multiple natural and built environments for months to years, presumably following contamination by a human source. Furthermore, several studies confirm *M. tuberculosis* viability and virulence in the environment using guinea pig and mouse models. Most of this evidence was historical; however, several recent studies have reported consistent findings of *M. tuberculosis* detection and viability in the environment using modern methods. Whether *M. tuberculosis* in environments represents an infectious threat to humans requires further investigation; this may represent an untapped source of data with which to further understand *M. tuberculosis* transmission. We discuss potential opportunities for harnessing these data to generate new insights into TB transmission in congregate settings. Copyright ©ERS 2019.

Lipworth, S., R. Jajou, A. De Neeling, P. Bradley, W. Van Der Hoek, G. Maphalala, M. Bonnet, E. Sanchez-Padilla, R. Diel, S. Niemann, Z. Iqbal, G. Smith, T. Peto, D. Crook, T. Walker and D. Van Soolingen (2019). "SNP-IT tool for identifying subspecies and associated lineages of *Mycobacterium tuberculosis* complex." *Emerging Infectious Diseases* 25(3): 482-488.

The clinical phenotype of zoonotic tuberculosis and its contribution to the global burden of disease are poorly understood and probably underestimated. This shortcoming is partly because of the inability of currently available laboratory and in silico tools to accurately identify all subspecies of the *Mycobacterium tuberculosis* complex (MTBC). We present SNPs to Identify TB (SNP-IT), a single-nucleotide polymorphism-based tool to identify all members of MTBC, including animal clades. By applying SNP-IT to a collection of clinical genomes from a UK reference laboratory, we detected an unexpectedly high number of *M. orygis* isolates. *M. orygis* is seen at a similar rate to *M. bovis*, yet *M.*

orygis cases have not been previously described in the United Kingdom. From an international perspective, it is possible that *M. orygis* is an underestimated zoonosis. Accurate identification will enable study of the clinical phenotype, host range, and transmission mechanisms of all subspecies of MTBC in greater detail. © 2019, Centers for Disease Control and Prevention (CDC). All rights reserved.

Kerr, T. J., C. R. de Waal, P. E. Buss, J. Hofmeyr, K. P. Lyashchenko and M. A. Miller (2019). "Seroprevalence of mycobacterium tuberculosis complex in free-ranging african elephants (*Loxodonta africana*) in Kruger national park, South Africa." *Journal of Wildlife Diseases* 55(4): 923-927.

Tuberculosis (TB) is a pathogenic disease that affects a range of wildlife species, including African elephants (*Loxodonta africana*). The recent discovery of fatal disease caused by infection with *Mycobacterium tuberculosis* in a bull elephant in the Kruger National Park (KNP), which is a bovine TB endemic area, emphasizes the importance this disease could have on both wild and captive elephant populations globally. Elephants with culture-confirmed TB have previously been shown to produce strong antibody-responses before the mycobacteria can be isolated. Therefore, we used two serologic assays that detect TB antibodies to retrospectively screen a cohort of 222 free-ranging African elephants sampled between 2004 and 2018 in KNP. The estimated TB seroprevalence for this free-roaming elephant population was between 6% (95% confidence interval [CI], 2–12%) and 9% (95% CI, 6–15%) based on the two tests. Overall, males had a higher TB seroprevalence than females, and adults (≤ 25 yr) had a higher TB seroprevalence than younger elephants (≤ 24 yr) on both rapid tests. The relatively high TB seroprevalence that we found highlighted the value of conducting retrospective studies in free-ranging wildlife populations in order to better understand the potential risk of disease. © Wildlife Disease Association 2019.

Yano, T., S. Premasathira, T. Dejyong, S. Tangtrongsup and M. D. Salman (2018). "The Effectiveness of a Foot and Mouth Disease Outbreak Control Programme in Thailand 2008-2015: Case Studies and Lessons Learned." *Vet Sci* 5(4).

Three Foot and Mouth Disease (FMD) outbreaks in northern Thailand that occurred during the implementation of the national FMD strategic plan in 2008(-)2015 are described to illustrate the lessons learned and to improve the prevention and control of future outbreaks. In 2008, during a FMD outbreak on a dairy farm, milk delivery was banned for 30 days. This was a part of movement management, a key strategy for FMD control in dairy farms in the area. In 2009, more than half the animals on a pig farm were affected by FMD. Animal quarantine and restricted animal movement played a key role in preventing the spread of FMD. In 2010, FMD infection was reported in a captive elephant. The suspected source of virus was a FMD-infected cow on the same premises. The infected elephant was moved to an elephant hospital that was located in a different province before the diagnosis was confirmed. FMD education was given to elephant veterinarians to promote FMD prevention and control strategies in this unique species. These three cases illustrate how differences in outbreak circumstances and species require the implementation of a variety of different FMD control and prevention measures. Control measures and responses should be customized in different outbreak situations.

Veerasami, M., K. Venkataraman, C. Karuppanan, A. A. Shanmugam, M. C. Prudhvi, T. Holder, P. Rathnagiri, K. Arunmozhivarman, G. D. Raj, M. Vordermeier and B. Mohana Subramanian (2018). "Point of Care Tuberculosis Sero-Diagnosis Kit for Wild Animals: Combination of Proteins for Improving the Diagnostic Sensitivity and Specificity." *Indian J Microbiol* 58(1): 81-92.

Tuberculosis is a significant problem globally for domestic animals as well as captive and free ranging wild life. Rapid point of care (POC) serology kits are well suited for the diagnosis of TB in wild animals. However, wild animals are invariably exposed to environmental non-pathogenic

mycobacterium species with the development of cross reacting antibodies. In the present study, POC TB diagnosis kit was developed using a combination of pathogenic Mycobacteria specific recombinant antigens and purified protein derivatives of pathogenic and non-pathogenic Mycobacteria. To benchmark the TB antibody detection kit, particularly in respect to specificity which could not be determined in wildlife due to the lack of samples from confirmed uninfected animals, we first tested well-characterized sera from 100 *M. bovis* infected and 100 uninfected cattle. Then we investigated the kit's performance using sera samples from wildlife, namely Sloth Bears (n = 74), Elephants (n = 9), Cervidae (n = 14), Felidae (n = 21), Cape buffalo (n = 2), Wild bear (n = 1) and Wild dog (n = 1). In cattle, a sensitivity of 81% and a specificity of 90% were obtained. The diagnostic sensitivity of the kit was 94% when the kit was tested using known TB positive sloth bear sera samples. 47.4% of the in-contact sloth bears turned seropositive using the rapid POC TB diagnostic kit. Seropositivity in other wild animals was 25% when the sera samples were tested using the kit. A point of care TB sero-diagnostic kit with the combination of proteins was developed and the kit was validated using the sera samples of wild animals.

Santos, N., T. Nunes, C. Fonseca, M. Vieira-Pinto, V. Almeida, C. Gortázar and M. Correia-Neves (2018). "Spatial analysis of wildlife tuberculosis based on a serologic survey using dried blood spots, Portugal." *Emerging Infectious Diseases* 24(12): 2169-2175.

We investigated the spatial epidemiology of bovine tuberculosis (TB) in wildlife in a multihost system. We surveyed bovine TB in Portugal by serologic analysis of elutes of dried blood spots obtained from hunted wild boar. We modeled spatial disease risk by using areal generalized linear mixed models with conditional autoregressive priors. Antibodies against *Mycobacterium bovis* were detected in 2.4% (95% CI 1.5%–3.8%) of 678 wild boar in 2 geographic clusters, and the predicted risk fits well with independent reports of *M. bovis* culture. Results show that elutes are an almost perfect substitute for serum (Cohen unweighted $\kappa = 0.818$), indicating that serologic tests coupled with dried blood spots are an effective strategy for large-scale bovine TB surveys, using wild boar as sentinel species. Results also show that bovine TB is an emerging wildlife disease and stress the need to prevent further geographic spread and prevalence increase. © 2018, Centers for Disease Control and Prevention (CDC). All rights reserved.

Riojas, M. A., K. J. McGough, C. J. Rider-Riojas, N. Rastogi and M. H. Hazbón (2018). "Phylogenomic analysis of the species of the mycobacterium tuberculosis complex demonstrates that mycobacterium africanum, mycobacterium bovis, mycobacterium caprae, mycobacterium microti and mycobacterium pinnipedii are later heterotypic synonyms of mycobacterium tuberculosis." *International Journal of Systematic and Evolutionary Microbiology* 68(1): 324-332.

The species within the *Mycobacterium tuberculosis* Complex (MTBC) have undergone numerous taxonomic and nomenclatural changes, leaving the true structure of the MTBC in doubt. We used next-generation sequencing (NGS), digital DNA–DNA hybridization (dDDH), and average nucleotide identity (ANI) to investigate the relationship between these species. The type strains of *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium microti* and *Mycobacterium pinnipedii* were sequenced via NGS. Pairwise dDDH and ANI comparisons between these, previously sequenced MTBC type strain genomes (including 'Mycobacterium canettii', 'Mycobacterium mungi' and 'Mycobacterium orygis') and *M. tuberculosis* H37RvT were performed. Further, all available genome sequences in GenBank for species in or putatively in the MTBC were compared to H37RvT. Pairwise results indicated that all of the type strains of the species are extremely closely related to each other (dDDH: 91.2–99.2 %, ANI: 99.21–99.92 %), greatly exceeding the respective species delineation thresholds, thus indicating that they belong to the same species. Results from the GenBank genomes indicate that all the strains examined are within the circumscription of

H37RvT (dDDH: 83.5–100 %). We, therefore, formally propose a union of the species of the MTBC as *M. tuberculosis*. *M. africanum*, *M. bovis*, *M. caprae*, *M. microti* and *M. pinnipedii* are reclassified as later heterotypic synonyms of *M. tuberculosis*. 'M. canettii', 'M. mungi', and 'M. orygis' are classified as strains of the species *M. tuberculosis*. We further recommend use of the infrasubspecific term 'variant' ('var.') and infrasubspecific designations that generally retain the historical nomenclature associated with the groups or otherwise convey such characteristics, e.g. *M. tuberculosis* var. *Bovis*. © 2018 by American Type Culture Collection (ATCC).

Paudel, S., S. K. Mikota, J. Thapa, K. P. Lyaschenko, K. P. Gairhe, I. P. Dhakal, N. Subedi, B. Maharjan, S. Subedi, G. E. Kaufman and T. Tsubota (2018). "Serodiagnosis of elephant tuberculosis: a useful tool for early identification of infected elephants at the captive-wild interface." *European Journal of Wildlife Research* 64: 70.

Tuberculosis (TB) is an emerging disease in elephants primarily caused by *Mycobacterium tuberculosis* (*M. tb*) and in some occasions by *M. bovis*. We performed culture and three serological tests—the Elephant TB STAT-PAK,[®] DPP VetTB[®] Assay, and MAPIA (multi-antigen print immunoassay)—prospectively on samples from eight elephants in Nepal that died of suspected or confirmed tuberculosis (TB) between 2007 and 2013. Among them, all elephants were reactive to DPP VetTB[®] Assay, five to Elephant TB STAT-PAK,[®] and two were reactive to MAPIA. Similarly, six elephants were positive on culture on samples collected antemortem or postmortem. We observed antibody responses months to years before culture confirmation of TB which shows that serological tests can be highly useful for the early diagnosis of TB in elephants. Validated point-of-care serological tests are easily performed in the field and hold promise for improved TB surveillance in other non-domestic species.

Miller, M. A., M. Finnegan, T. Storms, M. Garner and K. P. Lyashchenko (2018). "OUTBREAK OF MYCOBACTERIUM TUBERCULOSIS IN A HERD OF CAPTIVE ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*): ANTEMORTEM DIAGNOSIS, TREATMENT, AND LESSONS LEARNED." *J Zoo Wildl Med* 49(3): 748-754.

Tuberculosis (TB) was diagnosed in four Asian elephants (*Elephas maximus*) in a zoo in the United States. The first case was detected by isolation of *Mycobacterium tuberculosis* during routine trunk wash (TW) culture testing of a herd of eight elephants. Retrospective antibody analyses revealed seroconversion 1 yr before diagnosis. Serological testing of the whole elephant herd identified two additional suspect bulls with detectable antibody, but which remained culture-negative and had no clinical signs of disease. In the following months, *M. tuberculosis*, identical to the isolate from the index case, was isolated from TW samples of these two elephants. A fourth elephant seroconverted nearly 4 yr after the first TB case was detected, and *M. tuberculosis* was isolated from a TW sample collected 1 mo later. All four infected elephants received anti-TB therapy. Two treated elephants were eventually euthanized for reasons unrelated to *M. tuberculosis* and found to be culture-negative on necropsy, although one of them had PCR-positive lung lesions. One infected animal had to be euthanized due to development of a drug-resistant strain of *M. tuberculosis*; this animal did not undergo postmortem examination due to risk of staff exposure. The fourth animal is currently on treatment. Serial serological and culture results of the other four herd mates have remained negative.

Che-Amat, A. and B. L. Ong (2018). "Wildlife Tuberculosis in Southeast Asia: A Less Known Potential Hot-Spots and Issues in Disease Surveillance and Management." *Journal of Dairy and Veterinary Science* 6(2: 555683.).

Barandongo, Z. R., J. K. E. Mfuno and W. C. Turner (2018). "DUST-BATHING BEHAVIORS OF AFRICAN HERBIVORES AND THE POTENTIAL RISK OF INHALATIONAL ANTHRAX." *J Wildl Dis* 54(1): 34-44.

: Anthrax in herbivorous wildlife and livestock is generally assumed to be transmitted via ingestion or inhalation of *Bacillus anthracis* spores. Although recent studies have highlighted the importance of the ingestion route for anthrax transmission, little is known about the inhalational route in natural systems. Dust bathing could aerosolize soilborne pathogens such as *B. anthracis*, exposing dust-bathing individuals to inhalational infections. We investigated the potential role of dust bathing in the transmission of inhalational anthrax to herbivorous wildlife in Etosha National Park, Namibia, an area with endemic seasonal anthrax outbreaks. We 1) cultured soils from dust-bathing sites for the presence and concentration of *B. anthracis* spores, 2) monitored anthrax carcass sites, the locations with the highest *B. anthracis* concentrations, for evidence of dust bathing, including a site where a zebra died of anthrax on a large dust bath, and 3) characterized the ecology and seasonality of dust bathing in plains zebra (*Equus quagga*), blue wildebeest (*Connochaetes taurinus*), and African savanna elephant (*Loxodonta africana*) using a combination of motion-sensing camera traps and direct observations. Only two out of 83 dust-bath soils were positive for *B. anthracis*, both with low spore concentrations (≤ 20 colony-forming units per gram). We also detected no evidence of dust baths occurring at anthrax carcass sites, perhaps due to carcass-induced changes in soil composition that may deter dust bathing. Finally, despite observing some seasonal variation in dust bathing, preliminary evidence suggests that the seasonality of dust bathing and anthrax mortalities are not correlated. Thus, although dust bathing creates a dramatic cloud of aerosolized soil around an individual, our microbiologic, ecologic, and behavioral results in concert demonstrate that dust bathing is highly unlikely to transmit inhalational anthrax infections.

Veerasami, M., K. Venkataraman, C. Karuppanan, A. A. Shanmugam, M. C. Prudhvi, T. Holder, P. Rathnagiri, K. Arunmozhivarman, G. D. Raj, M. Vordermeier and B. Mohana Subramanian (2017). "Point of Care Tuberculosis Sero-Diagnosis Kit for Wild Animals: Combination of Proteins for Improving the Diagnostic Sensitivity and Specificity." *Indian Journal of Microbiology*: 1-12.

Tuberculosis is a significant problem globally for domestic animals as well as captive and free ranging wild life. Rapid point of care (POC) serology kits are well suited for the diagnosis of TB in wild animals. However, wild animals are invariably exposed to environmental non-pathogenic mycobacterium species with the development of cross reacting antibodies. In the present study, POC TB diagnosis kit was developed using a combination of pathogenic Mycobacteria specific recombinant antigens and purified protein derivatives of pathogenic and non-pathogenic Mycobacteria. To benchmark the TB antibody detection kit, particularly in respect to specificity which could not be determined in wildlife due to the lack of samples from confirmed uninfected animals, we first tested well-characterized sera from 100 *M. bovis* infected and 100 uninfected cattle. Then we investigated the kit's performance using sera samples from wildlife, namely Sloth Bears (n = 74), Elephants (n = 9), Cervidae (n = 14), Felidae (n = 21), Cape buffalo (n = 2), Wild bear (n = 1) and Wild dog (n = 1). In cattle, a sensitivity of 81% and a specificity of 90% were obtained. The diagnostic sensitivity of the kit was 94% when the kit was tested using known TB positive sloth bear sera samples. 47.4% of the in-contact sloth bears turned seropositive using the rapid POC TB diagnostic kit. Seropositivity in other wild animals was 25% when the sera samples were tested using the kit. A point of care TB sero-diagnostic kit with the combination of proteins was developed and the kit was validated using the sera samples of wild animals. © 2017 Association of Microbiologists of India

Liu, C., Z. Zhao, J. Fan, C. J. Lyon, H. J. Wu, D. Nedelkov, A. M. Zelazny, K. N. Olivier, L. H. Cazares, S. M. Holland, E. A. Graviss and Y. Hu (2017). "Quantification of circulating Mycobacterium tuberculosis antigen peptides allows rapid diagnosis of active disease and treatment monitoring." *Proceedings of the National Academy of Sciences of the United States of America* 114(15): 3969-3974.

Tuberculosis (TB) is a major global health threat, resulting in an urgent unmet need for a rapid, non-sputum-based quantitative test to detect active *Mycobacterium tuberculosis* (Mtb) infections in clinically diverse populations and quickly assess Mtb treatment responses for emerging drug-resistant strains. We have identified Mtb-specific peptide fragments and developed a method to rapidly quantify their serum concentrations, using antibody-labeled and energy-focusing porous discoidal silicon nanoparticles (nanodisks) and high-throughput mass spectrometry (MS) to enhance sensitivity and specificity. NanoDisk-MS diagnosed active Mtb cases with high sensitivity and specificity in a case-control study with cohorts reflecting the complexity of clinical practice. Similar robust sensitivities were obtained for cases of culture-positive pulmonary TB (PTB; 91.3%) and extrapulmonary TB (EPTB; 92.3%), and the sensitivities obtained for culture-negative PTB (82.4%) and EPTB (75.0%) in HIV-positive patients significantly outperformed those reported for other available assays. NanoDisk-MS also exhibited high specificity (87.1–100%) in both healthy and high-risk groups. Absolute quantification of serum Mtb antigen concentration was informative in assessing responses to antimycobacterial treatment. Thus, a NanoDisk-MS assay approach could significantly improve the diagnosis and management of active TB cases, and perhaps other infectious diseases as well. © 2017, National Academy of Sciences. All rights reserved.

Ghielmetti, G., M. Coscolla, M. Ruetten, U. Friedel, C. Loiseau, J. Feldmann, H. W. Steinmetz, D. Stucki and S. Gagneux (2017). "Tuberculosis in Swiss captive Asian elephants: microevolution of *Mycobacterium tuberculosis* characterized by multilocus variable-number tandem-repeat analysis and whole-genome sequencing." *Sci Rep* 7(1): 14647.

Zoonotic tuberculosis is a risk for human health, especially when animals are in close contact with humans. *Mycobacterium tuberculosis* was cultured from several organs, including lung tissue and gastric mucosa, of three captive elephants euthanized in a Swiss zoo. The elephants presented weight loss, weakness and exercise intolerance. Molecular characterization of the *M. tuberculosis* isolates by spoligotyping revealed an identical profile, suggesting a single source of infection. Multilocus variable-number of tandem-repeat analysis (MLVA) elucidated two divergent populations of bacteria and mixed infection in one elephant, suggesting either different transmission chains or prolonged infection over time. A total of eight *M. tuberculosis* isolates were subjected to whole-genome sequence (WGS) analysis, confirming a single source of infection and indicating the route of transmission between the three animals. Our findings also show that the methods currently used for epidemiological investigations of *M. tuberculosis* infections should be carefully applied on isolates from elephants. Moreover the importance of multiple sampling and analysis of within-host mycobacterial clonal populations for investigations of transmission is demonstrated.

Zlot, A., J. Vines, L. Nystrom, L. Lane, H. Behm, J. Denny, M. Finnegan, T. Hostetler, G. Matthews, T. Storms and E. DeBess (2016). "Diagnosis of Tuberculosis in Three Zoo Elephants and a Human Contact - Oregon, 2013." *MMWR Morb Mortal Wkly Rep* 64(52): 1398-1402.

In 2013, public health officials in Multnomah County, Oregon, started an investigation of a tuberculosis (TB) outbreak among elephants and humans at a local zoo. The investigation ultimately identified three bull elephants with active TB and 118 human contacts of the elephants. Ninety-six (81%) contacts were evaluated, and seven close contacts were found to have latent TB infection. The three bulls were isolated and treated (elephants with TB typically are not euthanized) to prevent infection of other animals and humans, and persons with latent infection were offered treatment. Improved TB screening methods for elephants are needed to prevent exposure of human contacts.

Young, L., S. Scott, M. Salfinger and E. Ramsay (2016). Serum concentrations of antimycobacterial drugs in Asian Elephants (*Elephas maximus*). AAZV / EAZWV / IZW Joint Conference.

Mycobacterium tuberculosis is an important disease of captive Asian elephants (*Elephas maximus*.) In this study six adult Asian elephants which had *Mycobacterium tuberculosis* cultured from trunk wash samples or had reactive DPP/MAPIA serologic responses were treated, concurrently, with one to three antimycobacterial drugs. Enrofloxacin hydrochloride, 2.5 mg/kg p.o., s.i.d., was administered to all animals in various foodstuffs for 9-15 mo. Serum enrofloxacin concentrations ranged from 230-2380 µg/ml (targeted concentrations = 125-1000 µg/ml).¹ Pyrazinamide (PZA), 30 mg/kg p.o., s.i.d., was administered to five elephants in various foodstuffs for 9-12 mo. Serum PZA concentrations ranged from 26-57 µg/ml (targeted concentrations = 20- 60 µg/ml).² Ethambutol (EMB), 30 mg/kg p.o., s.i.d., was administered to one elephant for 12 mo. A serum EMB concentration of 4.07 µg/ml was achieved (targeted concentration = 2-6 µg/ml).² Rifampin (RIF), 10 mg/kg p.o., s.i.d., was administered to one elephant for 9 mo. A serum RIF concentration of 16 µg/ml was achieved (targeted concentration = 8-24 µg/ml). All elephants were monitored for adverse clinical effects throughout treatments. Notable side effects were limited to excess, foamy lacrimation, believed to have occurred secondary to PZA administration. Clinical chemistries and complete blood counts were monitored in all animals and values remained within reference intervals throughout treatments. This study shows antimycobacterial drug dosages may require individuation, but concurrent, long-term, multidrug regimens for the treatment of *Mycobacterium tuberculosis* in Asian elephants can achieve appropriate therapeutic levels with minimal detrimental side effects.

Yakubu, Y., B. L. Ong, Z. Zakaria, L. Hassan, A. R. Mutalib, Y. F. Ngeow, K. Verasahib and M. F. Razak (2016). "Evidence and potential risk factors of tuberculosis among captive Asian elephants and wildlife staff in Peninsular Malaysia." *Prev Vet Med* 125: 147-153.

Elephant tuberculosis (TB) caused by *Mycobacterium tuberculosis* is an important re-emerging zoonosis with considerable conservation and public health risk. We conducted prospective cohort and cross-sectional studies in elephants and wildlife staff respectively in order to identify potential risk factors associated with TB in captive Asian elephants and their handlers in Peninsular Malaysia. Sixty elephants in six different facilities were screened for TB longitudinally using the ElephantTB STAT-PAK and DPP VetTB assays from February 2012 to May 2014, and 149 wildlife staff were examined for tuberculosis infection using the QuantiFERON-TB Gold In-tube (QFT) assay from January to April, 2012. Information on potential risk factors associated with infection in both elephants and staff were collected using questionnaires and facility records. The overall seroprevalence of TB amongst the elephants was 23.3% (95% CI: 13.8-36.3) and the risk of seroconversion was significantly higher among elephants with assigned mahouts [$p=0.022$, OR=4.9 (95% CI: 1.3-18.2)]. The percentage of QFT responders among wildlife staff was 24.8% (95% CI: 18.3-32.7) and the risk of infection was observed to be significantly associated with being a zoo employee [$p=0.018$, OR=2.7 (95% CI: 1.2-6.3)] or elephant handler [$p=0.035$, OR=4.1 (95% CI: 1.1-15.5)]. These findings revealed a potential risk of TB infection in captive elephants and handlers in Malaysia, and emphasize the need for TB screening of newly acquired elephants, isolating sero-positive elephants and performing further diagnostic tests to determine their infection status, and screening elephant handlers for TB, pre- and post-employment.

Steinmetz, H. and M. Rutten (2016). TB or Not TB: Diagnosis of tuberculosis in a group of Asian elephants (*Elephas maximus*). AAZV /EAZWV/IZW Joint Conference, Atlanta GA.

Animal and human health is inextricably interwoven; a good example is tuberculosis (TB). Although recognized as a disease of elephants for over 20 centuries, investigations into TB's prevalence in the captive Asian elephant (*Elephas maximus*) population only go back 20 yr.^{3,4} The increasing problem of human TB combined with the susceptibility of elephants and the close contact between human and elephant, makes surveillance based on reliable early diagnosis essential.³ Although the

availability of diagnostics for clinical applications has improved in recent years, there is still a wide discrepancy between their sensitivities and specificities.^{1,2}

In a group of 10 Asian elephants, tuberculosis was suspected from clinical observations and various clinical tests. Nevertheless, despite over 200 trunk washes being taken for analysis over a period of 14 mo, culture and RT-PCR tests for *M. tuberculosis* were negative. Three animals were euthanized due to severe geriatric health problems. Pathologic examination revealed typical *M. tuberculosis* lesions in lung and lymph nodes. Culture and RT-PCR performed from the lesions, of postmortem collected tracheal secretions and of stomach wall tissues confirmed *M. tuberculosis* infection.

Based on these results, utilization of a combination of clinical signs (e.g., chronic weight loss), standard tests (e.g., comparative intradermal tuberculin test, trunk wash culture or PCR) and newer serologic tests (e.g., sero-diagnostic tests - Dual Path Platform [DPP] VetTB and multiantigen print immunoassay [MAPIA]), and repeated testing to increase antemortem validity are recommended. Gastric and bronchial lavage should also be investigated to improve accuracy of antemortem diagnostics.

Paudel, S., M. A. Villanueva, S. K. Mikota, C. Nakajima, K. P. Gairhe, S. Subedi, N. Rayamajhi, M. Sashika, M. Shimozuru, T. Matsuba, Y. Suzuki and T. Tsubota (2016). "Development and evaluation of an interferon- γ release assay in Asian elephants (*Elephas maximus*)." *Journal of Veterinary Medical Science* 78(7): 1117-1121.

We developed an interferon- γ release assay (IGRA) specific for Asian elephants (*Elephas maximus*). Whole blood collected from forty captive Asian elephants was stimulated with three different mitogens i.e., phytohemagglutinin (PHA), pokweed mitogen (PWM) and phorbol myristate acetate/ionomycin (PMA/I). A sandwich ELISA that was able to recognize the recombinant elephant interferon- γ (rEIFN- γ) as well as native interferon- γ from the Asian elephants was performed using anti-elephant IFN- γ rabbit polyclonal antibodies as capture antibodies and biotinylated anti-elephant IFN- γ rabbit polyclonal antibodies as detection antibodies. PMA/I was the best mitogen to use as a positive control for an Asian elephant IGRA. The development of an Asian elephant-specific IGRA that detects native IFN- γ in elephant whole blood provides promising results for its application as a potential diagnostic tool for diseases, such as tuberculosis (TB) in Asian elephants. © 2016 The Japanese Society of Veterinary Science.

Paudel, S., J. L. Brown, S. Thapaliya, I. P. Dhakal, S. K. Mikota, K. P. Gairhe, M. Shimozuru and T. Tsubota (2016). "Comparison of cortisol and thyroid hormones between tuberculosis-suspect and healthy elephants of Nepal." *Journal of Veterinary Medical Science* 78(11): 1713-1716.

We compared cortisol and thyroid hormone (T3 and T4) concentrations between tuberculosis (TB)-suspected (n=10) and healthy (n=10) elephants of Nepal. Whole blood was collected from captive elephants throughout Nepal, and TB testing was performed using the ElephantTB STAT-PAK[®] and DPP VetTB[®] serological assays that detect antibodies against *Mycobacterium tuberculosis* and *M. bovis* in elephant serum. Cortisol, T3 and T4 were quantified by competitive enzyme immunoassays, and the results showed no significant differences in hormone concentrations between TB-suspect and healthy elephants. These preliminary data suggest neither adrenal nor thyroid function is altered by TB disease status. However, more elephants, including those positively diagnosed for TB by trunk wash cultures, need to be evaluated over time to confirm results. © 2016 The Japanese Society of Veterinary Science.

Hildebrandt, B., J. Saragusty, I. Moser, S. Holtze, T. Voracek, A. Bernhard, F. Goritz and R. Hermes (2016). Bronchialveolar lavage technique: a new approach for diagnosis of tuberculosis infection in elephants. *Joint AAZV / EAZWV / IZW*.

Tuberculosis in pachyderms was put into the spotlight two decades ago when circus elephants in North America were diagnosed with *Mycobacterium tuberculosis* complex. Because of the close association between elephants and humans, zoonotic risk, and high susceptibility to *Mycobacterium tuberculosis*, periodic testing was enacted in many zoological institutions around the world.^{1,2} Presently the gold standard is bacterial culture of trunk wash. Trunk wash, however, puts the operator at risk, it is insensitive, and is prone to contamination. We describe here a new technique that increases the safety and sensitivity while reducing the risk of cross-contamination. It was applied in one male and five female African and one male and three female Asian elephants. The technique relies on performing standing sedation with butorphanol 0.1 mg/kg combined with detomidine hydrochloride 0.02 mg/kg i.m. and additional nerve blocks in four locations to the trunk base 10 ml per location lidocaine hydrochloride 2%. A customized 3.5-m long videochip endoscope is inserted through the trunk and up to the larynx or the trachea. A sterile newly developed 6-hole-TBH-catheter named after inventor Thomas Bernd Hildebrandt with a length of 6 m is then placed through the 4 mm working channel of the endoscope further into the respiratory system. The lavage is performed using up to 100 ml sterile saline solution. Collection of the sample is done in closed system. The technique is safe for the operator, and has higher probability of harvesting the bacteria when such are shed while keeping environmental and trunk-related contamination to a minimum.

Abraham, D. and V. Pillai (2016). Cross-species transmission of *mycobacterium tuberculosis* in mahouts and captive elephants: Implications to health policy. 17th International Congress on Infectious Diseases / International Journal of Infectious Diseases

Background: There are nearly a thousand captive Asian elephants and not less than 3,000 mahouts in southern India. In the hands-on and open systems of captive elephant management, diseased mahouts and captive elephants could present the risk of cross-species tuberculosis transmission. With the help of evidence based results, we intend to formulate specific policy guidelines, which can suggest locally relevant preventive and control measures to help mitigate the risk of cross-species infection.

Methods & Materials: Over a period of three years, one time screening of nearly 800 elephants and their mahouts was achieved. Tuberculosis screening of mahouts was done by clinical examination, chest X-ray evaluation, sputum culture and tuberculin skin testing, as required. Screening of elephants was done using the USDA licensed serological test, DPP Vet Assay[®] (Chembio Diagnostics Inc., Medford, New York) and trunk wash culture, as required. Detailed contact investigation of traceable human and animal contacts of the identified diseased mahouts and elephants were done. We examined three different contexts of tuberculosis transmission among captive elephants and mahouts. First scenario is the risk of infection from an infected mahout to an elephant. Second is the risk of infection from an infected elephant to a mahout and third is the risk of infection from an infected elephant to another elephant.

Results: There is evidence to suggest cross-species tuberculosis transmission. However, under the tropical climatic conditions in southern India, the risk of infection to a captive elephant from a diseased mahout seems to far outweigh the risks of infection to a mahout or another elephant, from a diseased elephant. There are political as well as ethical consequences to the outcomes in each of the three scenarios and they are both varied and complex.

Conclusion: Mahouts and captive elephants in southern India are highly migrant and locating the subjects for contact tracing and follow-up testing is difficult. Hence, systematic and regular tuberculosis screening of mahouts and captive elephants is a challenge. Formulating as well as implementing policy guidelines for prevention and control of cross-species tuberculosis transmission, in the existing cultural and religious contexts of captive elephant managements in southern India, appears to be an even bigger challenge.

Vogelnest, L., F. Hulst, P. Thompson, K. P. Lyashchenko and K. A. Herrin (2015). "Diagnosis and management of tuberculosis (*Mycobacterium tuberculosis*) in an Asian elephant (*Elephas maximus*) with a newborn calf." *J Zoo Wildl Med* 46(1): 77-85.

In 2006, five Asian elephants (*Elephas maximus*) were imported to Taronga Zoo, Australia, from Thailand. Pre-import and initial postarrival tuberculosis screening was performed by trunk wash (TW) culture and was negative for *Mycobacterium tuberculosis*. In April 2009, the ElephantTB STAT-PAK (SP) assay was used to test the elephants. A 15.5-yr-old pregnant cow was reactive. TW frequency for this cow was increased from annually to quarterly. TW cultures remained negative on all other elephants. In February 2010, the Dual Path Platform (DPP) VetTB assay was used for the first time, and the SP-reactive cow also reacted on the DPP. A SP was run concurrently and was reactive. All other elephants were nonreactive on both assays. Treatment was not initiated due to concern about the effect of antituberculous drugs on the fetus. Quarterly TW cultures continued. The cow gave birth on 2 November 2010. A routine TW on 24 November 2010 was culture positive for *M. tuberculosis*. Although previous shedding could not be ruled out, reactivation of latent infection or exacerbation of subclinical disease due to parturition was suspected. Treatment with isoniazid, pyrazinamide, rifampicin, and ethambutol commenced. A 12-mo treatment course was completed within a 15-mo period. The isolate was susceptible to these drugs and genotyped as a Beijing strain. Stored serum samples from 2004 and 2006 were tested retrospectively and were reactive on SP and DPP. TW, SP, and DPP screening frequency increased to monthly for the positive cow on commencement of treatment in January 2011. Monthly serum biochemistry indicated drug-induced hepatitis. Therapeutic drug monitoring was conducted to ensure therapeutic levels were achieved. The infant calf was reactive on DPP, but TW culture negative, and was not treated. Serial DPP results for the cow and calf during and after treatment indicated that the antibody levels were declining, suggesting a favorable response to therapy in the dam, and that the origin of the antibodies in the calf were maternal, rather than a response to infection.

Perera, B. V. P., M. A. Salgado, G. S. P. d. S. Gunwardena, N. H. Smith and H. R. N. Jinadasa (2015). "First confirmed case of fatal tuberculosis in a wild Sri Lankan elephant." *Gajah* 41: 28-31.

Niemeier, R. T., K. Mead, M. A. dePerio, S. Martin and G. A. Burr (2015). Evaluation of Potential Employee Exposures to *Mycobacterium tuberculosis* at an Elephant Refuge, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health: 27.

Mikota, S. K., K. Gairhe, K. Giri, K. Hamilton, M. Miller, S. Paudel, K. Lyashchenko, R. S. Larsen, J. B. Payeur, W. R. Waters, R. Greenwald, G. Dumonceaux and B. Vincent (2015). "Tuberculosis surveillance of elephants (*Elephas maximus*) in Nepal at the captive-wild interface." *Eur J Wildl Res* 61: 221-229.

A comprehensive elephant tuberculosis (TB) survey using culture and four serological screening tests was conducted in Nepal in response to concern raised by wildlife officials that TB could threaten wild populations of elephants, rhinos, and other susceptible species. Captive elephants come into close contact with wild animals during conservation and tourism activities inside Nepal's national parks. Private and government-owned male and female captive Asian elephants (*Elephas maximus*) were included in the study. The mean reported age was 38 years (range 5-60 years). A total of 289 samples from 120 elephants were collected for mycobacterial culture. Culture samples were processed at the National Tuberculosis Centre (NTC) in Nepal and the National Veterinary Services Laboratories (NVSL) in Ames, IA. Acid-fast organisms were observed in 11 and 21 samples processed at NTC and NVSL, respectively, and nontuberculous mycobacteria (NTMs) were isolated from six elephants. There were

no isolations of *Mycobacterium tuberculosis* or *Mycobacterium bovis*. Blood samples were also collected from 115 of the elephants for serological testing using the Chembio ElephantTB STAT-PAK[®], the Chembio MultiAntigen Print Immunoassay test, a multi-antigen ELISA, and an immunoblot assay. Culture and serological results were variable and required careful interpretation to develop criteria to assess TB risk. Elephants were assigned to one of four disease risk groups (high, moderate, low, and undetermined), and management recommendations for each group were made to government authorities. Serological results were prioritized in developing recommendations because of culture limitations and inconclusive culture results. This strategy was based on evidence for the early predictive value of serological tests and the urgent need expressed by wildlife authorities in Nepal to protect their captive elephants, mitigate TB at the captive-wild interface, and safeguard tourism.

Lassausaie, J., A. Bret, X. Bouapao, V. Chanthavong, J. Castonguay-Vanier, F. Quet, S. K. Mikota, C. Theoret, Y. Buisson and B. Bouchard (2015). "Tuberculosis in Laos, who is at risk: the mahouts or their elephants?" *Epidemiol Infect* 143(5): 922-931.

SUMMARY Tuberculosis (TB) in elephants has the potential to infect humans and is an increasing public health concern. Lao PDR is one of the last countries where elephants are still used for timber extraction and where they live in close contact with their mahouts. There are 500 animals at work in the country, some interacting with wild herds. Although human TB prevalence is known to be high in Laos, studies on elephant TB had yet to be undertaken. From January to July 2012, screening was performed using the ElephantTB Stat-Pak assay on 80 elephants working around the Nam Pouy National Park in Sayaboury Province. This represents more than 18% of the total registered national working elephant population. Here we report that 36% of the elephants were seroreactive to the test. Of these, 31% had contacts with wild individuals, which suggests potential transmission of mycobacteria to the local wild herds. Clinical examination, chest X-rays, sputum microscopy and culture were performed on their 142 mahouts or owners. Despite high TB seroreactivity in elephants, no participant was smear- or culture-positive for *Mycobacterium tuberculosis* or *M. bovis*, although atypical mycobacteria were isolated from 4% of participants.

Lassausaie, J., A. Bret, X. Bouapao, V. Chanthavong, J. Castonguay-Vanier, F. Quet, S. K. Mikota, C. Theoret, Y. Buisson and B. Bouchard (2014). "Tuberculosis in Laos, who is at risk: the mahouts or their elephants?" *Epidemiol Infect* 143(5): 922-931.

Tuberculosis (TB) in elephants has the potential to infect humans and is an increasing public health concern. Lao PDR is one of the last countries where elephants are still used for timber extraction and where they live in close contact with their mahouts. There are 500 animals at work in the country, some interacting with wild herds. Although human TB prevalence is known to be high in Laos, studies on elephant TB had yet to be undertaken. From January to July 2012, screening was performed using the ElephantTB Stat-Pak assay on 80 elephants working around the Nam Pouy National Park in Sayaboury Province. This represents more than 18% of the total registered national working elephant population. Here we report that 36% of the elephants were seroreactive to the test. Of these, 31% had contacts with wild individuals, which suggests potential transmission of mycobacteria to the local wild herds. Clinical examination, chest X-rays, sputum microscopy and culture were performed on their 142 mahouts or owners. Despite high TB seroreactivity in elephants, no participant was smear- or culture-positive for *Mycobacterium tuberculosis* or *M. bovis*, although atypical mycobacteria were isolated from 4% of participants.

Hlokwe, T. M., P. van Helden and A. L. Michel (2014). "Evidence of increasing intra and inter-species transmission of *Mycobacterium bovis* in South Africa: Are we losing the battle?" *Preventive Veterinary Medicine* 115(1-2): 10-17.

Tuberculosis caused by *Mycobacterium bovis* is recognized worldwide as a significant health risk in domestic cattle, farmed and wild animal species as well as in humans. We carried out spoligotyping and variable number of tandem repeat (VNTR) typing methods to characterize 490 *M. bovis* isolates from livestock (cattle, n= 230; pig n= 1) and wildlife species (n= 259) originating from different farms and regions in South Africa, with the aim to further establish the genetic diversity of the isolates, study the population structure of *M. bovis* and elucidate the extent of interspecies transmission of bovine tuberculosis. A total of ten spoligotype patterns were identified, two of which were novel (SB2199 and SB2200) and reported for the first time in the literature, while VNTR typing revealed a total of 97 VNTR profiles. Our results showed evidence of clonal expansion for some ancestral strains as well as co-infections with two or three *M. bovis* strains on some of the cattle and game farms, which suggested independent introductions of infected animals from epidemiologically unrelated sources. Five spoligotypes and nine VNTR profiles were shared between cattle and wildlife. Our findings showed that besides cattle, at least 16 different animal species in South Africa are infected with bovine tuberculosis, and highlight a strong evidence of inter and intra-species transmission of *M. bovis*. Infection of the blue wildebeest (*Connochaetes taurinus*) with *M. bovis* is described for the first time in this report. This update in epidemiological information raises concerns that bovine tuberculosis has increased its spatial distribution in South Africa and is also affecting an increasing number of wildlife species compared to ten years ago. © 2014 Elsevier B.V.

van Sandwyk, J. H., N. C. Bennett, R. Swanepoel and A. D. S. Bastos (2013). "Retrospective genetic characterisation of Encephalomyocarditis viruses from African elephant and swine recovers two distinct lineages in South Africa." *Veterinary Microbiology* 162(1): 23-31.

Encephalomyocarditis virus (EMCV) outbreaks are rare in southern Africa. Only two have been reported to date from South Africa, both coinciding with rodent irruptions. The first outbreak manifested as acute myocarditis in pigs in 1979, whilst the second, occurring from 1993 to 1994, was linked to the deaths of 64 free-ranging adult African elephants (*Loxodonta africana*). The P1 genome region, inclusive of the flanking leader (L) and 2A genes, of three South African isolates, one from swine and two from elephants, was characterised by PCR amplification and sequencing of up to 11 overlapping fragments. In addition to the resulting 3329 nucleotide dataset, the 3D region that is widely used in molecular epidemiology studies, was characterised, and three datasets (P1, VP1/3 and 3D), complemented with available homologous EMCV data, were compiled for analyses. Phylogenetic inferences revealed the near-identical elephant outbreak strains to be most closely related to a mengovirus from rhesus macaques (*Macaca mulatta*) in Uganda, differing from the latter by between 11% (3D) and 15% (VP3/1). The South African pig isolate differed by 4% (3D) and 11% (VP3/1) from available European and Asian pig virus sequences. This study confirms the presence of two genetically distinct EMCV lineages recovered from sporadic outbreaks in wild and domestic hosts in southern Africa, and provides valuable baseline data for future outbreak eventualities in the sub-region. © 2012 Elsevier B.V.

Ong, B. L., Y. F. Ngeow, M. F. Razak, Y. Yakuba, Z. Zakaria, A. R. Mutalib, L. Hassan, H. F. Ng and K. Versahib (2013). "Tuberculosis in captive Asian elephants (*Elephas maximus*) in peninsular Malaysia." *Epidemiol Infect*(141): 1481-1487.

A cross-sectional study was conducted from 10 January to 9 April 2012, to determine the seroprevalence of tuberculosis (TB) of all captive Asian elephants and their handlers in six locations in Peninsular Malaysia. In addition, trunk-wash samples were examined for tubercle bacillus by culture and polymerase chain reaction (PCR). For 63 elephants and 149 elephant handlers, TB seroprevalence was estimated at 20.4% and 24.8%, respectively. From 151 trunk-wash samples, 24 acid-fast isolates were obtained, 23 of which were identified by hsp65-based sequencing as non-tuberculous

mycobacteria. The Mycobacterium tuberculosis-specific PCR was positive in the trunk-wash samples from three elephants which were also seropositive. Conversely, the trunk wash from seven seropositive elephants were PCR negative. Hence, there was evidence of active and latent TB in the elephants and the high seroprevalence in the elephants and their handlers suggests frequent, close contact, two-way transmission between animals and humans within confined workplaces.

Obanda, V., J. Poghon, M. Yongo, M. Mulei, M. Ngotho, K. Waititu, J. Makumi, F. Gakuya, P. Osmondi, R. C. Soriguer and S. Alasaad (2013). "First reported case of fatal tuberculosis in a wild African elephant with past human-wildlife contact." *Epidemiol Infect* 141: 1476-1480.

Tuberculosis is emerging/re-emerging in captive elephant populations, where it causes morbidity and deaths, although no case of TB in wild African elephants has been reported. In this paper we report the first case of fatal TB in an African elephant in the wild. The infection with Mycobacterium tuberculosis was confirmed by post-mortem and histological examinations of a female sub-adult elephant aged >12 years that died in Tsavo East National Park, Kenya, while under treatment. This case is unique in that during its lifetime the elephant had contact with both humans and wild elephants. The source of the infection was unclear because the elephant could have acquired the infection in the orphanage or in the wild. However, our results show that wild elephants can maintain human TB in the wild and that the infection can be fatal.

Miller, M. and F. Olea-Popelka (2013). "One Health in the shrinking world: Experiences with tuberculosis at the human-livestock-wildlife interface." *Comparative Immunology Microbiology and Infectious Diseases* 36(3): 263-268.

Tuberculosis (TB) is a global anthroozoonotic infection that has raised awareness of the impact of disease at the human-livestock-wildlife interface. There are examples of transmission from livestock resulting in establishment of reservoirs in wildlife populations, and exposures from interactions between humans and wildlife that have resulted in disease outbreaks. A One Health approach is crucial to managing and protecting the health of humans, livestock, wildlife and the environment. Although still in its infancy in many areas of the world, the use of transdisciplinary teams to address wildlife-human-livestock boundary diseases will broaden the scope of options for solutions. This paper reviews some less commonly known examples of threats and outcomes using lessons learned from tuberculosis. (C) 2012 Elsevier Ltd. All rights reserved.

Mikota, S. K., S. Subedi, K. Gairhe, S. Paudel, J. Thapa, B. Vincent and G. Kaufman (2013). Nepal elephant (*Elephas maximus*) Healthcare and Tuberculosis Surveillance Program Update. American Association of Zoo Veterinarians.

The Nepal Elephant Healthcare and Tuberculosis (TB) Surveillance Program was initiated by Elephant Care International in 2007 following the first comprehensive TB testing of Asian elephants in 2006. Previous reports have described the challenges that TB presents to wildlife, humans, and domestic livestock in Nepal 1-3 and a recent report has demonstrated the risk of transmission to the wild.⁴

The program is based near Chitwan National Park where a field office and lab are staffed by a full-time veterinarian. Program goals are to 1) mitigate transmission of TB to wild elephants, rhinos and other ungulates by controlling TB at the captive-wild interface, 2) ensure the health of government elephants used for anti-poaching patrols, rhino censuses, and other conservation purposes, 3) safeguard tourism that supports the national parks, 4) build wildlife veterinary capacity, 5) encourage the development of elephant TB control programs other Asian elephant range countries, and 6) advance our knowledge of TB in elephants.

Ninety-three percent of the captive population has been tested using the Elephant TB Stat-Pak® and / or DPP® Vet TB™ assays. Over 20 elephants have been treated prophylactically or therapeutically for TB based on serology results, culture, and /or exposure history.

The Program has facilitated multiple research projects, involving students and investigators from Tufts University, Michigan State University, Murdoch University, and the Institute of Agriculture and Animal Science (Nepal).

In 2010 the Ministry of Forestry approved the Elephant Tuberculosis Control and Management Action Plan (2011-2015), the first such plan in Asia. The plan is on-line at www.elephantcare.org.

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We greatly acknowledge the financial support of the U.S Fish and Wildlife Services Asian Elephant Conservation Fund (Awards 98201-8-G571, 96200-9-G222, and 96200-0-G143), the Mazuri Fund, the Walter J. Ernst Memorial Fund, the Abraham Foundation, Buttonwood Park Zoo, Columbus Zoo, Oklahoma City Zoo, Phoenix Zoo, Busch Gardens Tamps, the Humane Society of the United States, and numerous private donors.

Products Mentioned in the Text: Chembio Diagnostic Systems, Inc, Medford, NY, USA 11763.

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Feldman, M., R. Isaza, C. Prins and J. Hernandez (2013). "Point prevalence and incidence of Mycobacterium tuberculosis complex in captive elephants in the United States of America." *Veterinary Quarterly* 33: 25-29.

Angkawanish, T., D. Morar, P. van Kooten, I. Bontekoning, J. Schreuder, M. Maas, W. Wajjwalku, A. Sirimalaisuwan, A. Michel, E. Tijhaar and V. Rutten (2013). "The elephant interferon gamma assay: a contribution to diagnosis of tuberculosis in elephants." *Transbound Emerg Dis* 60 Suppl 1: 53-59.

Mycobacterium tuberculosis (*M. tb*) has been shown to be the main causative agent of tuberculosis in elephants worldwide. *M. tb* may be transmitted from infected humans to other species including elephants and vice versa, in case of prolonged intensive contact. An accurate diagnostic approach covering all phases of the infection in elephants is required. As *M. tb* is an intracellular pathogen and cell-mediated immune (CMI) responses are elicited early after infection, the skin test is the CMI assay of choice in humans and cattle. However, this test is not applicable in elephants. The interferon gamma (IFN-gamma) assay is considered a good alternative for the skin test in general, validated for use in cattle and humans. This study was aimed at development of an IFN-gamma assay applicable for diagnosis of tuberculosis in elephants. Recombinant elephant IFN-gamma (rEpIFN-gamma) produced in eukaryotic cells was used to immunize mice and generate the monoclonal antibodies. Hybridomas were screened for IFN-gamma-specific monoclonal antibody production and subcloned, and antibodies were isotyped and affinity purified. Western blot confirmed recognition of the rEpIFN-gamma. The optimal combination of capture and detection antibodies selected was able to detect rEpIFN-gamma in concentrations as low as 1 pg/ml. The assay was shown to be able to detect the native elephant IFN-gamma, elicited in positive-control cultures (pokeweed mitogen (PWM), phorbol myristate acetate plus ionomycin (PMA/I)) of both Asian and African elephant whole-blood cultures (WBC). Preliminary data were generated using WBC from non-infected elephants, a *M. tb* infection-suspected elephant and a culture-confirmed *M. tb*-infected elephant. The latter showed measurable production of IFN-gamma after stimulation with ESAT6/CFP10 PPDB and PPDA in concentration ranges as elicited in WBC by *Mycobacterium tuberculosis* complex (MTBC)-specific antigens in other species. Hence, the IFN-gamma assay presented potential as a diagnostic tool for the detection of elephant tuberculosis. Validation of the assay will require its application in large populations of non-infected and infected elephants.

Verma-Kumar, S., D. Abraham, N. Dendukuri, J. V. Cheeran, R. Sukumar and K. N. Balaji (2012). "Serodiagnosis of tuberculosis in Asian elephants (*Elephas maximus*) in southern India: a latent class analysis." *PLoS ONE* 7(11): 1-8.

Lyashchenko, K. P., R. Greenwald, J. Esfandiari, S. Mikota, M. Miller, T. Moller, L. Volgelnest, K. Gairhe, S. Robbe-Austerman, J. Gai and W. R. Waters (2012). "Field application of serodiagnostics to identify elephants with tuberculosis prior to case confirmation by culture." *Clinical and Vaccine Immunology* 19(8): 1269-1275.

Three serologic methods for antibody detection in elephant tuberculosis (TB), the multiantigen print immunoassay (MAPIA), ElephantTB STAT-PAK kit, and DPP VetTB test, were evaluated using serial serum samples from 14 captive elephants infected with *Mycobacterium tuberculosis* in 5 countries. In all cases, serological testing was performed prior to the diagnosis of TB by mycobacterial culture of trunk wash or tissue samples collected at necropsy. All elephants produced antibody responses to *M. tuberculosis* antigens, with 13/14 recognizing ESAT-6 and/or CFP10 proteins. The findings supported the high serodiagnostic test accuracy in detecting infections months to years before *M. tuberculosis* could be isolated from elephants. The MAPIA and/or DPP VetTB assay demonstrated the potential for monitoring antimycobacterial therapy and predicting TB relapse in treated elephants when continuously used in the posttreatment period. History of exposure to TB and past treatment information should be taken into consideration for proper interpretation of the antibody test results. Data suggest that the more frequent trunk wash culture testing of seropositive elephants may enhance the efficiency of the TB diagnostic algorithm, leading to earlier treatment with improved outcomes.

Yong, H., C. Go-Eun, B. S. Lee, J. Whang and S. J. Shin (2011). "Disseminated infection due to *Mycobacterium avium* subsp. *avium* in an Asian elephant (*Elephas maximus*." *Journal of Zoo and Wildlife Medicine* 42(4): 743-746.

Witt, C. J., A. L. Richards, P. M. Masuoka, D. H. Foley, A. L. Buczak, L. A. Musila, J. H. Richardson, M. G. Colacicco-Mayhugh, L. M. Rueda, T. A. Klein, A. Anyamba, J. Small, J. A. Pavlin, M. M. Fukuda, J. Gaydos, K. L. Russell, A.-G. P. S. W. Group, R. C. Wilkerson, R. V. Gibbons, R. G. Jarman, K. S. Myint, B. Pendergast, S. Lewis, J. E. Pinzon, K. Collins, M. Smith, E. Pak, C. Tucker, K. Linthicum, T. Myers, M. Mansour, K. Earhart, H. C. Kim, J. Jiang, D. Schnabel, J. W. Clark, R. C. Sang, E. Kioko, D. C. Abuom, J. P. Grieco, E. E. Richards, S. Tobias, M. R. Kasper, J. M. Montgomery, D. Florin, J. P. Chretien and T. L. Philip (2011). "The AFHSC-Division of GEIS Operations Predictive Surveillance Program: a multidisciplinary approach for the early detection and response to disease outbreaks." *BMC Public Health* 11 Suppl 2: S10.

The Armed Forces Health Surveillance Center, Division of Global Emerging Infections Surveillance and Response System Operations (AFHSC-GEIS) initiated a coordinated, multidisciplinary program to link data sets and information derived from eco-climatic remote sensing activities, ecologic niche modeling, arthropod vector, animal disease-host/reservoir, and human disease surveillance for febrile illnesses, into a predictive surveillance program that generates advisories and alerts on emerging infectious disease outbreaks. The program's ultimate goal is pro-active public health practice through pre-event preparedness, prevention and control, and response decision-making and prioritization. This multidisciplinary program is rooted in over 10 years experience in predictive surveillance for Rift Valley fever outbreaks in Eastern Africa. The AFHSC-GEIS Rift Valley fever project is based on the identification and use of disease-emergence critical detection points as reliable signals for increased outbreak risk. The AFHSC-GEIS predictive surveillance program has formalized the Rift Valley fever project into a structured template for extending predictive surveillance capability to other Department of Defense (DoD)-priority vector- and water-borne, and zoonotic diseases and geographic areas. These include leishmaniasis, malaria, and Crimea-Congo and other viral hemorrhagic fevers in Central Asia and Africa, dengue fever in Asia and the Americas, Japanese encephalitis (JE) and chikungunya fever in Asia, and rickettsial and other tick-borne infections in the U.S., Africa and Asia.

Murphree, R., J. V. Warkentin, J. R. Dunn, W. Schaffner and T. F. Jones (2011). "Elephant-to-human transmission of tuberculosis, 2009." *Emerg Infect Dis* 17(3): 366-371.

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.

Mikota, S. K. and J. N. Maslow (2011). "Tuberculosis at the human-animal interface: an emerging disease of elephants." *Tuberculosis (Edinb)* 91(3): 208-211.

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.

Giri, K., G. E. Kaufman and I. P. Dhakal (2011). "The relationship between blood parameter and mycobacterium culture status in captive elephants of Nepal." *Nepalese Vet J* 30: 1190-1120.

Sang, R., E. Kioko, J. Lutomiah, M. Warigia, C. Ochieng, M. O'Guinn, J. S. Lee, H. Koka, M. Godsey, D. Hoel, H. Hanafi, B. Miller, D. Schnabel, R. F. Breiman and J. Richardson (2010). "Rift Valley fever virus epidemic in Kenya, 2006/2007: the entomologic investigations." *Am J Trop Med Hyg* 83(2 Suppl): 28-37.

In December 2006, Rift Valley fever (RVF) was diagnosed in humans in Garissa Hospital, Kenya and an outbreak reported affecting 11 districts. Entomologic surveillance was performed in four districts to determine the epidemic/epizootic vectors of RVF virus (RVFV). Approximately 297,000 mosquitoes were collected, 164,626 identified to species, 72,058 sorted into 3,003 pools and tested for RVFV by reverse transcription-polymerase chain reaction. Seventy-seven pools representing 10 species tested positive for RVFV, including *Aedes mcintoshi/circumluteolus* (26 pools), *Aedes ochraceus* (23 pools), *Mansonia uniformis* (15 pools); *Culex poicilipes*, *Culex bitaeniorhynchus* (3 pools each); *Anopheles squamosus*, *Mansonia africana* (2 pools each); *Culex quinquefasciatus*, *Culex univittatus*, *Aedes pembaensis* (1 pool each). Positive *Ae. pembaensis*, *Cx. univittatus*, and *Cx. bitaeniorhynchus* was a first time observation. Species composition, densities, and infection varied among districts supporting hypothesis that different mosquito species serve as epizootic/epidemic vectors of RVFV in diverse ecologies, creating a complex epidemiologic pattern in East Africa.

Michel, A. L., B. Muller and P. D. van Helden (2010). "Mycobacterium bovis at the animal-human interface: A problem of not?" *Veterinary Microbiology* 140: 371-381.

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.

Landolfi, J. A., S. K. Mikota, J. Chosy, K. P. Lyaschenko, K. Giri, K. Gairhe and K. A. Terio (2010). "Comparison of systemic cytokine levels in *Mycobacterium* spp seropositive and seronegative Asian elephants (*Elephas maximus*)." *Journal of Zoo and Wildlife Medicine* 41(3): 445-455.

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.

Ireton, G. C., R. Greenwald, H. Liang, J. Esfandiari, K. P. Lyashchenko and S. G. Reed (2010). "Identification of *Mycobacterium tuberculosis* antigens of high serodiagnostic value." *Clinical and Vaccine Immunology* 17(10): 1539-1547.

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis*, with several million new cases detected each year. Current methods of diagnosis are time-consuming and/or expensive or have a low level of accuracy. Therefore, new diagnostics are urgently needed to address the global tuberculosis burden and to improve control programs. Serological assays remain attractive for use in resource-limited settings because they are simple, rapid, and inexpensive and offer the possibility of detecting cases often missed by routine sputum smear microscopy. The aim of this study was to identify *M. tuberculosis* seroreactive antigens from a panel of 103 recombinant proteins selected as diagnostic candidates. Initial library screening by protein array analysis and enzyme-linked immunosorbent assay (ELISA) identified 42 antigens with serodiagnostic potential. Among these, 25 were novel proteins. The reactive antigens demonstrated various individual sensitivities, ranging from 12% to 78% (specificities, 76 to 100%). When the antigens were analyzed in combinations, up to 93% of antibody responders could be identified among the TB patients. Selected seroreactive proteins were used to design 3 new polyepitope fusion proteins. Characterization of these antigens by multiantigen print immunoassay (MAPIA) revealed that the vast majority of the TB patients (90%) produced antibody responses. The results confirmed that due to the remarkable variation in immune recognition patterns, an optimal multiantigen cocktail should be designed to cover the heterogeneity of antibody responses and thus achieve the highest possible test sensitivity. Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Angkawanish, T., W. Wajjwalku, A. Sirimalaisuwan, S. Mahasawangkul, T. Kaewsakhorn, K. Boonsri and V. P. M. G. Rutten (2010). "Mycobacterium tuberculosis infection of domesticated Asian elephants, Thailand." *Emerg Infect Dis* 16(12): 1949-1951.

Alexander, K. A., P. N. Laver, A. L. Michel, M. Williams, P. D. van Helden, R. M. Warren and N. C. G. van Pittius (2010). "Novel mycobacterium tuberculosis complex pathogen, *M. Mungi*." *Emerging Infectious Diseases* 16(8): 1296-1299.

Seven outbreaks involving increasing numbers of banded mongoose troops and high death rates have been documented. We identified a *Mycobacterium tuberculosis* complex pathogen, *M. mungi* sp. nov., as the causative agent among banded mongooses that live near humans in Chobe District, Botswana. Host spectrum and transmission dynamics remain unknown.

Mikota, S. K., G. Kaufman, I. P. Dhakal and B. D. Pandey (2009). Tuberculosis in Nepal: Elephants, Humans, Livestock, and Wildlife. *Proceedings of the American Association of Zoo Veterinarians*.

Tuberculosis (TB) is endemic among humans in Nepal. Almost 50% of the > 28 million population are infected and up to 90,000 are active cases (<http://www.who-int/infnew/tuber4.htm>). Direct observed therapy short-course (DOTS) was instituted in 1996 and now reaches 75% of the population. Implementation of DOTS nation-wide is hampered by the logistics of reaching and servicing remote hill areas. Between 5,000 and 7,000 people die every year despite DOTS therapy; some of these deaths may be due to multidrug-resistant (MDR) or extensively drug-resistant (XDR) TB. Four drug resistance surveys have been carried out since 2005. MDR-TB rates of 2.9% (1.8%-3.2%) among new cases and 11.7% (7.1%-18.3%) among re-treatment cases were reported at the end of the fourth survey (http://www.searo.who.int/en/Section10/Section2097/Section2100_14801.htm).

Nepal has a mixed farming system, including over four million buffaloes and almost seven million cattle. Sporadic studies have identified a TB prevalence of 0-24% among cattle and 4.5 to 41% among buffalo. In a recent study *Mycobacterium bovis* (*M. bovis*) was isolated from 17% of buffalo and 16% of cattle positive on the single intradermal cervical test.¹ There is no formal TB surveillance or control program for cattle or buffalo in Nepal. Although the World Health Organization recommends test and slaughter to eliminate bovine TB, Nepal is predominantly Hindu and the slaughter of cattle is forbidden.

The prevalence of *M. bovis* (BTB) infection in humans is unknown as TB diagnostic laboratories in Nepal (as in many countries) report positive culture results as "*M. tuberculosis* complex" but do not speciate. Risks of TB / BTB transmission from livestock to people exist through direct contact by farmers and slaughterhouse workers and consumption of contaminated meat and unpasteurized milk. Buffalo meat comprises over 64% of the total meat consumed in Nepal. In one study, tuberculosis was diagnosed in 14% of slaughtered buffaloes.² Intensive livestock production is rare, and human beings live in close association with their farm animals providing increased opportunities for exposure.

Captive elephants in Nepal are cared for by humans, bred by wild elephant bulls, and graze with domestic livestock. Government-owned elephants patrol the Chitwan National Park (and other protected areas) and are essential for rhino counts and other conservation programs. Privately owned elephants used for safaris in the parks generate tourist dollars that support conservation and local businesses.

TB has not yet been diagnosed in wild elephants, rhinos, or other wild mammals in Nepal but poses a significant threat. Controlling TB at the captive elephant interface may decrease transmission to the wild where it would be difficult if not impossible to control. An elephant TB surveillance program was initiated in Nepal in 2006 following the postmortem diagnosis of TB in several captive elephants. To date, 164 captive elephants (79% of the population) have been tested using the ElephantTB STAT-PAK Assay® (Chembio Diagnostic Systems, Inc., 3661 Horseblock Road, Medford, NY 11763, USA). Nineteen elephants are receiving treatment for TB; one elephant has completed treatment, and one old elephant is under permanent quarantine. Culture-confirmation of TB infection has been unrewarding due to 1) difficulty in performing the trunk wash procedure, 2) sample contamination, and 3) limited laboratory capacity to process elephant samples. Investigation of alternative direct methods for diagnosis are being pursued.³ TB has not been detected in currently employed elephant caretakers tested by the public health system.

Tuberculosis will be a main focus of the newly established One Health-Nepal, spearheaded by the National Trust for Nature Conservation (a Nepal NGO) and the Zoological Society of London. Elephant Care International, the Cummings School of Veterinary Medicine at Tufts University, and the Institute of Agriculture and Animal Science are among the organizations that will collaborate to address cross-species TB issues in Nepal.

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2. Joshi, D.D. 1986. Epidemiological situation of tuberculosis in Nepal. *J. Inst. Med.* 5: 115-128.
3. Wilson, T., D. Akiyoshi, S. Desai, M. Bhandar, S. Paudel, P. Manandhar, S. Manandhar, S. Mikota, J. Mukherjee, and G. Kaufman. 2008. Development of a PCR diagnostic technique for differentiation of Mycobacterium species in elephant trunk wash samples in Nepal. Poster AAZV Annual Conference, Los Angeles, October 12-17, 2008

Mikota, S. K. (2009). Stress, Disease, and Tuberculosis in Elephants. *An Elephant in the Room*. D. L. Forthman, L. F. Kane, D. Hancocks and P. F. Waldau. North Grafton, Center for Animals and Public Policy, Cummings School of Veterinary Medicine, Tufts University: 74-84.

Michel, A. L., M. L. Coetzee, D. F. Keet, L. Mare, R. Warren, D. Cooper, R. G. Bengis, K. Kremer and P. van Helden (2009). "Molecular epidemiology of Mycobacterium bovis isolates from free-ranging wildlife in South African game reserves." *Vet Microbiol* 133: 335-343.

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.

Greenwald, R., O. Lyashchenko, J. Esfandiari, M. Miller, S. Mikota, J. H. Olsen, R. Ball, G. Dumonceaux, D. Schmitt, T. Moller, J. B. Payeur, B. Harris, D. Sofranko, W. R. Waters and K. P. Lyashchenko (2009). "Highly accurate antibody assays for early and rapid detection of tuberculosis in African and Asian elephants." *Clin Vaccine Immunol* 16(5): 605-612.

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

Chambers, M. A. (2009). "Review of the diagnosis and study of tuberculosis in non-bovine wildlife species using immunological methods." *Transboundary and Emerging Diseases* 56: 215-227.

Mikota, S. K. (2008). "Review of tuberculosis in captive elephants and implications for wild populations." *Gajah* 28: 8-18.

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

(2008) Guidelines for the control of tuberculosis in elephants.

Une, Y. and T. Mori (2007). "Tuberculosis as a zoonosis from a veterinary perspective." *Comp Immunol Microbiol Infect Dis* 30: 415-425.

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.

Mikota, S. K., M. Miller, G. Dumonceaux, K. Giri, K. Gairhe, K. Hamilton, S. Paudel, K. Lyashchenko, R. S. Larsen, J. Payeur, R. Waters, M. D. Salman and G. Kaufman (2007). Comparison of four serological tests and culture to determine tuberculosis infection in captive elephants in Nepal. Proceedings AAZV,AAWV,AZA/NAG Joint Conference.

Hamilton, K., S. K. Mikota, M. Miller, G. Dumonceaux, K. Giri, K. Gairhe, S. Paudel and G. Kaufman (2007). Evaluation of blood chemistry values for possible relationship to tuberculosis infection status in captive elephants in (*Elephas maximus*) Nepal. Proceedings AAZV,AAWV,AZA/NAG Joint Conference.

One hundred fifteen captive elephants (*Elephas maximus*) were examined in Nepal as part of a tuberculosis (TB) survey in January 2006. Blood chemistry analysis was performed at Disney's Animal Kingdom laboratory (USA). Trunk wash cultures were performed both in Nepal and in the USA, and serologic TB tests were performed in the USA. Based on culture and serology results, the elephants were grouped as follows: Group 1 (high risk, suggestive or confirmatory for TB infection) and Group 2 (low risk, equivocal or negative for TB infection). Within these groups, subgroups were created based on specific tests results. Blood chemistry results were analyzed to reveal any relationships between these values and TB infection status. Student t-tests were performed on each value between Groups 1 and 2. The only significant difference was a higher BUN/creatinine ratio ($p=0.047$) in Group 1. ANOVA analysis was performed on each value between the various groups. Significant differences were found in the albumin level ($p=0.015$) within the Group 1 subgroups and in the albumin level ($p=0.002$), alpha globulin 1 level ($p=0.030$), and A/G ratio ($p=0.012$) within the Group 2 subgroups.

This study did not reveal an association between certain chemistry values and TB infection. However, this may be due to a variety of age, reproductive statuses, stages of infections, and other possible medical conditions. Future testing of this population will help better define the TB infection status of elephants and may provide additional information to more precisely determine any association between blood chemistry values and tuberculosis infection in Nepal elephants.

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research contributions from Konstantin Lyashchenko, Dr. Scott Larsen, Dr. Janet Payeur, and Dr. Ray Waters.

Rothschild, B. M. and L. D. Martin (2006). "Did ice-age bovids spread tuberculosis?" *Naturwissenschaften* 93: 565-569.

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. and R. Laub (2006). "Hyperdisease in the late Pleistocene: validation of an early 20th century hypothesis." *Naturwissenschaften* 93: 557-564.

Peloquin, C. A., J. N. Maslow, S. K. Mikota, A. Forrest, F. Dunker, R. Isaza, L. R. Peddie, J. Peddie and M. Zhu (2006). "Dose selection and pharmacokinetics of rifampin in elephants for the treatment of tuberculosis." *Journal of Veterinary Pharmacology and Therapeutics* 29(6): 581-585.

Moller, T., B. O. Roken, S. S. Lewerin and K. Lyashchenko (2006). "The elephant Rapid Test (RT) the future diagnostic test for TB (*M. tuberculosis*) in elephants? Call for a validation study in Europe." *Proceedings International Elephant Conservation and Research Symposium*: 119-124.

Mikota, S. K., M. Miller, G. Dumonceaux, K. Giri, K. Gairhe, K. Hamilton, S. Paudel and B. Vincent (2006). Elephant tuberculosis diagnosis: implications for elephant management in Asian range countries. 2006 *Proceedings American Association of Zoo Veterinarians*.

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 (culturepositive) 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.

Mikota, S. K., G. Dumonceaux, M. Miller, K. Gairhe, K. Giri, J. V. Cheeran, D. Abraham, K. Lyashchenko, S. Larsen, J. Payeur, R. Waters, G. Kaufman and \ (2006). "Tuberculosis in elephants: An update on diagnosis and treatment; implications for control in range countries." *Proceedings International Elephant Conservation and Research Symposium*: 109-118.

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

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.

Lyashchenko, K. P., R. Greenwald, J. Esfandiari, J. H. Olsen, R. Ball, G. Dumonceaux, F. Dunker, C. Buckley, M. Richard, S. Murray, J. B. Payeur, P. Andersen, J. M. Pollock, S. Mikota, M. Miller, D. Sofranko and W. R. Waters (2006). "Tuberculosis in elephants: antibody responses to defined antigens of *Mycobacterium tuberculosis*, potential for early diagnosis, and monitoring of treatment." *Clin. Vaccine Immunol* 13(7): 722-732.

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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Ball, R. L., G. Dumonceaux, J. H. Olsen, M. S. Burton and K. Lyashchenko (2006). 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.

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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Zhu, M., J. N. Maslow, S. K. Mikota, R. Isaza, F. Dunker, H. Riddle and C. A. Peloquin (2005). "Population pharmacokinetics of pyrazinamide in elephants." J. Vet. Pharmacol. Ther 28(5): 403-409.

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

Waters, W. R., M. V. Palmer, J. P. Bannantine, R. Greenwald, J. Esfandiari, P. Andersen, J. McNair, J. M. Pollock and K. P. Lyashchenko (2005). "Antibody responses in reindeer (*Rangifer tarandus*) infected with Mycobacterium bovis." Clinical and Diagnostic Laboratory Immunology 12(6): 727-735.

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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Lyashchenko, K., M. Miller and W. R. Waters (2005). 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.

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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Lewerin, S. S., S. L. Olsson, K. Eld, B. Roken, S. Ghebremichael, T. Koivula, G. Kallenius and G. Bolske (2005). "Outbreak of *Mycobacterium tuberculosis* infection among captive Asian elephants in a Swedish zoo." *Vet. Rec* 156(6): 171-175.

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*.

Larsen, R. S., M. Kay, J. Triantis and M. D. Salman (2005). Update on serological detection of *Mycobacterium tuberculosis* infection in Asian elephants. 2005 Proceedings AAZV, AAWV, AZA Nutrition Advisory Group.

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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Lacasse, C., K. C. Gamble, K. Terio, L. L. Farina, D. A. Travis and M. Miller (2005). *Mycobacterium szulgai* osteoarthritis and pneumonia in an African elephant (*Loxodonta Africana*). 2005 Proceedings AAZV, AAWV, AZA Nutrition Advisory Group.

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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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.

Stringfield, C. E., P. Oh, R. Granich, J. Scott, B. Sun, M. Joseph, J. Flood and C. J. Sedgwick (2004). Epidemiologic investigation of a *Mycobacterium tuberculosis* infection of multiple animal species in a metropolitan zoo. 2004 PROCEEDINGS AAZV, AAWV, WDA JOINT CONFERENCE.

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 euthanatized 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 euthanatized 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

The authors thank the Animal Care and Animal Health staff of the Los Angeles Zoo who cared so well for these animals, and the veterinarians (including consulting pathologists), technicians, and medical records staff who collected, analyzed, and organized the clinical data. We could not have performed this evaluation without Sue Thisdell, Safety Officer at the Los Angeles Zoo; Jothan Staley and Donna Workman-Malcom of the City of Los Angeles Occupational Health Services Division; Lee Borenstein, Elenor Lehnkering, Patrick Ryan, Jeanne Soukup, and Annette Nita of the Los Angeles County Department of Health Services; and Diana Whipple for her RFLP expertise.

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Kaneene, J. B. and C. Thoen (2004). "Tuberculosis." JAVMA 224(5): 685-691.

Janssen, D. L., J. E. Oosterhuis, J. Fuller and K. Williams (2004). 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.

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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Ziccardi, M., H. N. Wong, L. A. Tell, D. Fritcher, J. Blanchard, A. Kilbourn and H. P. Godfrey (2003). Further optimization and validation of the antigen 85 immunoassay for diagnosing mycobacteriosis in wildlife. *Proc Amer Assoc Zoo Vet*.

Mycobacteriosis caused by *Mycobacterium bovis*, *M. tuberculosis* and *M. avium* has been a well-documented health problem for zoological collections as long ago as the late 19th century. Prevalence estimation in these captive wildlife populations, however, has been hampered by diagnostic test methods that are oftentimes difficult or impossible to conduct and/or interpret (due to the requirement for multiple immobilizations for measurement of response), the occurrence of non-specific results with methods such as the intradermal skin test, and/or the near-total lack of validation, optimization and standardization of any of the available test methods in the species of interest. Additionally, because intradermal skin testing is the primary screening method for many of these species, the ability to compare exposure in captive wildlife with exposure in free-ranging populations has been limited due to the difficulty with follow-up in free-ranging populations. Lastly, unlike testing methods that use serological techniques, skin testing precludes retrospective studies of banked samples to determine onset of reactivity.

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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3. Mangold, B. J., R. A. Cook, M. R. Cranfield, K. Huygen, and H. P. Godfrey. 1999. Detection of elevated levels of circulating antigen 85 by dot immunobinding assay in captive wild animals with tuberculosis. *J. Zoo Wildl. Med.* 30(4): 477-483.

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

Potters, D., M. Seghers, G. Muyldermans, D. Pie´rard, A. Naessens and S. Lauwers (2003). "Recovery of *Mycobacterium elephantis* from sputum of a patient in Belgium." *Journal of Clinical Microbiology* 41(3): 1344-1344.

Mycobacterium elephantis was isolated from a human respiratory specimen in April 1999, demonstrating its presence in Europe. The biochemical reaction results, antimicrobial susceptibility pattern, and sequence data for this strain are all in agreement with those of *M. elephantis* strains isolated previously from other continents.

Pavlik, I., W. Y. Ayele, I. Parmova, I. Melicharek, M. Hanzlikova, M. Svejnochova and B. Kormendy (2003). "Mycobacterium tuberculosis in animal and human populations in six Central European countries during 1990-1999." *Veterinarni Medicina* 48(4): 83-89.

Results of *Mycobacterium tuberculosis* detection in animals from six Central European countries (Croatia, the Czech Republic, Hungary, Poland, Slovakia and Slovenia) spreading over 610402 km² with a population of 11.8 million heads of cattle were analysed. In the monitoring period between 1990 and 1999, *M. tuberculosis* from animals was isolated only in two countries (Poland and Slovak Republic) from 16 animals with tuberculous lesions. These comprise 9 cattle (*Bos taurus*), 4 domestic pigs (*Sus scrofa f. domestica*) and three wild animals, an African elephant (*Loxodonta africana*), agouti (*Dasyprocta aguti*) and terrestrial tapir (*Tapirus terrestris*) from a zoological garden Gdansk in Poland. A steady decrease in the incidence of tuberculosis in humans was recorded during the monitoring period in all countries. The human population of the study countries was 68.03 million. In the period monitored, infection caused by *M. tuberculosis* was identified in a total of 241040 patients with a decreasing incidence of tuberculosis found in all countries. The lowest relative bacteriologically confirmed disease was found in the Czech Republic, Slovak Republic and Slovenia. Given the low number of infected domestic and wild animals, the epidemiological and epizootiological situation may be considered auspicious.

Michel, A. L., L. Venter, I. W. Espie and M. L. Coetzee (2003). "Mycobacterium tuberculosis infections in eight species at the National Zoological Gardens of South Africa, 1991-2001." *Journal of Zoo and Wildlife Medicine* 34(4): 364-370.

Between 1991 and 2001 a total of 12 cases of *Mycobacterium tuberculosis* infection in eight different species were recorded in the National Zoological Gardens of South Africa in Pretoria (Tshwane). The genetic relatedness between seven of the *M. tuberculosis* isolates was determined by IS6110 restriction fragment length polymorphism analysis. For the majority of the isolates that were analyzed, a high degree of polymorphism suggested different sources of infection. Evidence of *M. tuberculosis* transmission between animals is reported in two chimpanzees (*Pan troglodytes*) housed together, from which samples were collected for analysis 29 mo apart.

Chakraborty, A. (2003). "Diseases of elephants (*Elephas maximus*) in India-A Review." *Indian Wildlife Year Book* 2: 74-82.

(2003) Guidelines for the control of tuberculosis in elephants.

Turenne, C., P. Chedore, J. Wolfe, F. Jamieson, K. May and A. Kabani (2002). "Phenotypic and molecular characterization of clinical isolates of *Mycobacterium elephantis* from human specimens." *J Clin Microbiol* 40(4): 1230-1236.

Eleven strains of a rapidly growing mycobacterium were isolated from patient specimens originating from various regions of the province of Ontario, Canada, over a 2-year period. Unique high-performance liquid chromatography (HPLC) and PCR-restriction enzyme pattern analysis (PRA) profiles initially suggested a new *Mycobacterium* species, while sequencing of the 16S rRNA gene revealed a sequence match with *Mycobacterium* sp. strain MCRO 17 (GenBank accession no. X93028), an isolate determined to be unique which is to date uncharacterized, and also a close similarity to *M. elephantis* (GenBank accession no. AJ010747), with six base pair variations. A complete biochemical profile of these isolates revealed

a species of mycobacteria with phenotypic characteristics similar to those of *M. flavescens*. HPLC, PRA, and 16S rRNA sequencing of strain *M. elephantis* DSM 44368(T) and result comparisons with the clinical isolates revealed that these strains were in fact *M. elephantis*, a newly described species isolated from an elephant. All strains were isolated from human samples, 10 from sputum and 1 from an axillary lymph node.

Peloquin, C. (2002). "Therapeutic drug monitoring in the treatment of tuberculosis." *Drugs* 62(15): 2169-2183.

Payeur, J. B., J. L. Jarnagin, J. G. Marquardt and D. L. Whipple (2002). "Mycobacterial isolations in captive elephants in the United States." *Ann N Y Acad Sci* 969: 256-258.

Interest in tuberculosis in elephants has been increasing over the past several years in the United States. Several techniques have been used to diagnose mammalian tuberculosis. Currently, the test considered most reliable for diagnosis of TB in elephants is based on the culture of respiratory secretions obtained by trunk washes.

Oh, P., R. Granich, J. Scott, B. Sun, M. Joseph, C. Stringfield, S. Thisdell, J. Staley, D. Workman-Malcolm, L. Borenstein, E. Lehnkering, P. Ryan, J. Soukup, A. Nitta and J. Flood (2002). "Human exposure following *Mycobacterium tuberculosis* infection of multiple animal species in a Metropolitan Zoo." *Emerg Infect Dis* 8(11): 1290-1293.

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.

Mikota, S. K. and J. Maslow (2002). *Epidemiology and Treatment of Tuberculosis in Elephants: 2002*. American Association of Zoo Veterinarians Annual Conference.

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

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

The earliest writing describing the diseases of elephants in ancient literature said to be the works on "Gajasastra" (Elephantology) written in Sanskrit by authors like Gautama, Narada,

Mrigacharma, Rajaputra and Vyasa. "Hasthyayurveda" a legendary book in Sanskrit written by a sage Palakapya deals with some diseases, treatment, desirable and undesirable points of selection, management practices and some mythological aspects on the origin of elephants. The earliest book in English dealing with diseases of elephants seems to be that of W. Gilchrist "A practical treatise on the treatment of diseases of elephants" published in 1848. Later Slym (1873), Sanderson (1878), Steel (1885), Evans (1910), Herpburn (1913), Milroy (1922), Ptaff (1940), Ferrier (1947), Utoke Gale (1974), Chandrasekharan (1979) and Panicker (1985) have documented their findings on the incidence, etiology and control of diseases of Asian elephants.

Auclair, B., S. Mikota, C. A. Peloquin, R. Aguilar and J. N. Maslow (2002). "Population pharmacokinetics of antituberculous drugs and treatment of *Mycobacterium bovis* infection in Bongo Antelope (*Tragelaphus eurycrus isaaci*)." *Journal of Zoo and Wildlife Medicine* 33(3): 193-203.

Alexander, K. A., E. Pleydell, M. C. Williams, E. P. Lane, J. F. C. Nyange and A. L. Michel (2002). "Mycobacterium tuberculosis: An Emerging Disease of Free-Ranging Wildlife." *Emerging Infectious Diseases* 8(6): 598-601.

Expansion of ecotourism-based industries, changes in land-use practices, and escalating competition for resources have increased contact between free-ranging wildlife and humans. Although human presence in wildlife areas may provide an important economic benefit through ecotourism, exposure to human pathogens may represent a health risk for wildlife. This report is the first to document introduction of a primary human pathogen into free-ranging wildlife. We describe outbreaks of *Mycobacterium tuberculosis*, a human pathogen, in free-ranging banded mongooses (*Mungos mungo*) in Botswana and suricates (*Suricata suricatta*) in South Africa. Wildlife managers and scientists must address the potential threat that humans pose to the health of free-ranging wildlife.

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

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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Davis, M. (2001). "Mycobacterium tuberculosis risk for elephant handlers and veterinarians." *Appl Occup Environ Hyg* 16(3): 350-353.

Clifton-Hadley, R. S., C. M. Sauter-Louis, I. W. Lugton, R. Jacson, P. A. Durr and J. W. Wilesmith (2001). *Mycobacterial diseases. Infectious Diseases of Wild Mammals*. E. S. Williams. Ames, Iowa, Iowa State University Press,: 340-361.

Ziccardi, M., S. K. Mikota, R. B. Barbiere and T. M. Norton (2000). Tuberculosis in zoo ungulates: Survey results and surveillance plan. *Proc. AAZV and IAAAM Joint Conf.*

Shojaei, H., J. G. Magee, R. Freeman, M. Yates, N. U. Horadagoda and M. Goodfellow (2000). "Mycobacterium elephantis sp. nov., a rapidly growing non-chromogenic *Mycobacterium* isolated from an elephant." *International Journal of Systematic and Evolutionary Microbiology* 50(5): 1817-1820.

A strain isolated from a lung abscess in an elephant that died from chronic respiratory disease was found to have properties consistent with its classification in the genus *Mycobacterium*. An almost complete sequence of the 16S rDNA of the strain was determined following the cloning and sequencing of the amplified gene. The sequence was aligned with those available on mycobacteria and phylogenetic trees inferred by using three tree-making algorithms. The organism, which formed a distinct phyletic line within the evolutionary radiation occupied by rapidly growing mycobacteria, was readily distinguished from members of validly described species of rapidly growing mycobacteria on the basis of its mycolic acid pattern and by a number of other phenotypic features, notably its ability to grow at higher temperatures. The type strain is *Mycobacterium elephantis* DSM 44368T. The EMBL accession number for the 16S rDNA sequence of strain 484T is AJ010747.

Mikota, S. K., R. S. Larsen and R. J. Montali (2000). "Tuberculosis in Elephants in North America." *Zoo Biol* 19: 393-403.

Within the past 4 years, TB has emerged as a disease of concern in elephants. The population of elephants in North America is declining (Weise, 1997), and transmissible diseases such as TB may exacerbate this trend. Guidelines for all elephants for TB, were instituted in 1997 (USDA, 1997, 2000). Between August 1996 and May 2000, *Mycobacterium tuberculosis* was isolated from 18 of 539 elephants in North America, indicating an estimated prevalence of 3.3%. Isolation of the TB organism by culture is the currently recommended test to establish a diagnosis of TB; however, culture requires 8 weeks. Further research is essential to validate other diagnostic tests and treatment protocols.

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

Larsen, R. S., M. D. Salman, S. K. Mikota, R. Isaza and J. Triantis (2000). Validation and use of a multiple-antigen ELISA for detection of tuberculosis infections in elephants. *Proc. AAZV and IAAAM Joint Conf.*

Larsen, R. S., M. D. Salman, S. K. Mikota, R. Isaza, R. J. Montali and J. Triantis (2000). "Evaluation of a multiple-antigen enzyme-linked immunosorbent assay for detection of Mycobacterium tuberculosis infection in captive elephants." *Journal of Zoo and Wildlife Medicine* 31(3): 291-302.

Mycobacterium tuberculosis has become an important agent of disease in the captive elephant population of the United States, although current detection methods appear to be inadequate for effective disease management. This investigation sought to validate a multiple-antigen enzyme-linked immunosorbent assay (ELISA) for screening of M. tuberculosis infection in captive elephants and to document the elephant's serologic response over time using a cross-sectional observational study design. Serum samples were collected from 51 Asian elephants (*Elephas maximus*) and 26 African elephants (*Loxodonta africana*) from 16 zoos and circuses throughout the United States from February 1996 to March 1999. Infection status of each animal was determined by mycobacterial culture of trunk washes. Reactivity of each serum sample against six antigens was determined, and the linear combination of antigens that accurately predicted the infection status of the greatest number of 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.

Boomershine, C. S. and B. S. Zwilling (2000). "Stress and the pathogenesis of tuberculosis." *Clinical Microbiology Newsletter* 22(23): 177-182.

Mikota, S. K. (1999). "Diseases of the Elephant: A Review." *Verh. ber. Erkr. Zootiere* 39: 1-15.

Mangold, B. J., R. A. Cook, M. R. Cranfield, K. Huygen and H. P. Godfrey (1999). "Detection of elevated levels of circulating antigen 85 by dot immunobinding assay in captive wild animals with tuberculosis." *Journal of Zoo and Wildlife Medicine* 30(4): 477-483.

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.

Isaza, R. and C. J. Ketz (1999). "A Trunk Wash Technique for the Diagnosis of Tuberculosis in Elephants." *Verh. ber. Erkr. Zootiere* 39: 121-124.

Biberstein, E. L. and D. C. Hirsch (1999). *Mycobacterium species: The agents of animal tuberculosis*. Veterinary Microbiology. Maiden, MA, Blackwell Science: 158-172.

Bhat, M. N., R. Manickam and J. Ramkrishna (1999). "Screening of captive wild animals for tuberculosis." *Indian Veterinary Journal* 76(11): 959-961.

The passive haemagglutination (PHA) test was used to test 109 captive elephants (*Elephas maximus*), and spotted deer (*Cervus axis*), blackbuck (*Antelope cervicapra*) and common langurs (*Semnopithecus entellus*?) (4 of each) for tuberculosis; 51 of the elephants and the 4 langurs were also assessed by the tuberculin test. PHA titres of 1:16 or 1:32 were found in 4 elephants, 1 deer and 2 langurs, but all were apparently healthy except 1 langur that had clinical signs indicative of tuberculosis. There were 4 positive reactors in the tuberculin tests, all elephants, but these animals did not have significant PHA titres. It is concluded that the procedures and reagents used for the diagnosis of tuberculosis in domestic animals are not reliable for testing wild animals.

Montali, R. J., L. H. Spelman, R. C. Cambre, D. Chatterjee and S. K. Mikota (1998). Factors influencing interpretation of indirect testing methods for tuberculosis in elephants. Proceedings AAZV and AAWV Joint Conference.

Serologic and other laboratory tests (such as BTB, ELISA, and gamma interferon) are often used in conjunction with the intradermal tuberculin test to detect tuberculosis (TB) in animals. The skin test is considered the "gold standard" in domestic cattle and humans, and the BTB test has been highly rated for use in cervid species. However, these indirect methods for TB diagnosis have not been proven valid in most exotic species susceptible to *Mycobacterium tuberculosis* complex (which includes *M. bovis*) infection. In addition, many of the tuberculin skin testing methods used in exotic species are not uniform in terms of tuberculin type(s) and sites used and interpretation of the endpoints.

Michalak, K., C. Austin, S. Diesel, M. J. Bacon, P. Zimmerman and J. N. Maslow (1998). "Mycobacterium tuberculosis infection as a zoonotic disease: transmission between humans and elephants." *Emerg Infect Dis* 4(2): 283-287.

Between 1994 and 1996, three elephants from an exotic animal farm in Illinois died of pulmonary disease due to *Mycobacterium tuberculosis*. In October 1996, a fourth living elephant was culture-positive for *M. tuberculosis*. Twenty-two handlers at the farm were screened for tuberculosis (TB); eleven had positive reactions to intradermal injection with purified protein derivative. One had smear-negative, culture-positive active TB. DNA fingerprint comparison by IS6110 and TBN12 typing showed that the isolates from the four elephants and the handler with active TB were the same strain. This investigation indicates transmission of *M. tuberculosis* between humans and elephants.

Mahato, G., H. Rahman, K. K. Sharma and S. C. Pathak (1998). "Tuberculin testing in captive Indian elephants (*Elephas maximus*) of a national park." *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* 19(1): 63.

Full text: Tuberculosis, an important zoonotic disease, has been reported in wild African and Asian domestic elephants (Seneviratna and Seneviratna, 1966). Under this communication 25 captive Indian elephants of Kaziranga National Park, Assam, were tested for allergic reaction by injecting 0.1 ml PPD at the base of ear tip. The thickness of skin was measured after 48 and 72 h and an increase of 4 mm or more was taken as positive. Out of 25 elephants tested, 3 adults were found reactors. Base of the ear was found more appropriate site as it remained protected from rubbing against hard object due to irritation caused by the tuberculin and needle. The trunk also could not disturb this inoculation site.

Dunker, F. and M. Rudovsky (1998). Management and treatment of a *Mycobacterium tuberculosis* positive elephant at the San Francisco Zoo. Proceedings AAZV and AAWV Joint Conference.

Anonymous (1998). "TB in elephants." *Communique* 18.

Whipple, D. L., R. M. Meyer, D. F. Berry, J. L. Jarnagin and J. B. Payeur (1997). Molecular epidemiology of tuberculosis in wild white-tailed deer in Michigan and elephants. Proceedings One Hundred and First Annual Meeting of the United States Animal Health Association, United States Animal Health Association.

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

Peloquin, C. (1997). "Using therapeutic drug monitoring to dose the antimycobacterial drugs." *Clinics in Chest Medicine* 18: 79-97.

Mikota, S. K. and J. Maslow (1997). Theoretical and technical aspects of diagnostic techniques for mammalian tuberculosis. Proceedings, American Association Zoo Veterinarians.

Maslow, J. (1997). Tuberculosis and other mycobacteria as zoonoses. Proceedings American Association of Zoo Veterinarians.

Mycobacterial infections are common among humans. Of these, infection with *Mycobacterium tuberculosis* (TB) is the most common and of greatest concern. Non-tuberculous species of mycobacteria may also cause infections in man, especially among immunosuppressed individuals. Human TB is typically acquired by inhalation of aerosols carrying tubercle bacilli following exposure to a person with active pulmonary infection; non-tuberculous species of mycobacteria are acquired from environmental sources. Since zoonotic transmission of TB does occur, the identification of acid fast bacilli (AFB) in clinical specimens from animals is a cause of concern, unease, and occasionally misconception for animal care handlers and zoo personnel.

Furley, C. W. (1997). "Tuberculosis in elephants." *Lancet British edition* 350(9072): 224.

Tests on 171 elephants in zoos and circuses in the USA revealed that 33% were positive to one or more skin tests and 11% were positive by ELISA. As there is no standard procedure for testing elephants caution should be used when interpreting the results.

Essey, M. A. and J. P. Davis (1997). Status of the National cooperative state-federal bovine tuberculosis eradication program fiscal year 1997. Proceedings United States Animal Health Association.

Binkley, M. (1997). Tuberculosis in captive elephants. Proceedings American Association of Zoo Veterinarians.

Sandin, R. L. (1996). "Polymerase chain reaction and other amplification techniques in mycobacteriology." *Clinical Mycobacteriology* 16(3): 617-639.

Moda, G., C. J. Daborn, J. M. Grange and O. Cosivi (1996). "The zoonotic importance of *Mycobacterium bovis*." *Tubercle and Lung Disease* 77: 103-108.

The zoonotic importance of *Mycobacterium bovis* has been the subject of renewed interest in the wake of the increasing incidence of tuberculosis in the human population. This paper considers some of the conditions under which transmission of *M. bovis* from animals to humans occurs and reviews current information on the global distribution of the disease. The paper highlights the particular threat posed by this zoonotic disease in developing countries and lists the veterinary and human public health measures that need to be adopted if the disease is to be contained. The association of tuberculosis with malnutrition and poverty has long been recognized and the need to address these basic issues as crucial as specific measures against the disease itself.

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

Chandrasekharan, K., K. Radhakrishnan, J. V. Cheeran, K. N. M. Nair and T. Prabhakaran (1995). Review of the Incidence, Etiology and Control of Common Diseases of Asian Elephants with Special Reference to Kerala. *A Week with Elephants; Proceedings of the International Seminar on Asian Elephants*. J. C. Daniel. Bombay, India, Bombay Natural History Society; Oxford University Press: 439-449.

Incidence, etiology, symptoms and control of specific and non-specific diseases of captive and wild elephants have been reviewed. Asian elephants have been observed to be susceptible to various parasitic diseases such as helminthiasis, trypanosomiasis and ectoparasitic infestations, bacterial diseases such as tetanus, tuberculosis, haemorrhagic septicemia, salmonellosis and anthrax, viral diseases such as foot and mouth disease, pox and rabies and non-specific diseases like impaction of colon, foot rot and corneal opacity. A detailed study extending over two decades on captive and wild elephants in Kerala, revealed high incidence of helminthiasis (285), ectoparasitic infestation (235), impaction of colon (169) and foot rot (125). Diseases such as trypanosomiasis (21), tetanus (8), tuberculosis (5) pox (2) and anthrax (1) were also encountered. The line of treatment against the diseases mentioned, have been discussed in detail.

(1994). "Treatment of tuberculosis and tuberculosis infection in adults and children." *Am J Respir Crit Care Med* 149: 1359-1374.

Chandrasekharan, K. (1992). Prevalence of infectious diseases in elephants in Kerala and their treatment. *The Asian Elephant: Ecology, Biology, Diseases, Conservation and Management* (Proceedings of the National Symposium on the Asian Elephant held at the Kerala Agricultural University, Trichur, India, January 1989). E. G. Silas, M. K. Nair and G. Nirmalan. Trichur, India, Kerala Agricultural University: 148-155.

John, M. C., S. Nedunchelliyar and N. Raghavan (1991). "Tuberculin testing in Indian elephants." *Indian Journal of Veterinary Medicine* 11(1-2): 48-49.

Fowler, M. E. (1991). Tuberculosis in zoo ungulates. Bovine tuberculosis in cervidae: Proceedings of a symposium, United States Department of Agriculture Miscellaneous Publication No. 1506.

Sabin, J. E. (1990). "Joseph Hersey Pratt's cost-effective class method and its contemporary application." *Psychiatry* 53: 169-184.

Haagsma, J. and A. Eger (1990). ELISA for diagnosis of tuberculosis and chemotherapy in zoo and wildlife animals.

The aim of this study was to improve the diagnosis of bovine tuberculosis in zoo and wildlife animals, in particular by using an Enzyme-Linked Immunosorbent Assay (ELISA). In addition, suspected cases of tuberculosis (TB) with a positive skin test and /or ELISA were treated with antituberculosis drugs. The diagnosis of TB in animals is based primarily on the intradermal tuberculin test, corresponding with cellular immune response. Although this test has practical disadvantages in zoo animals, the application is still of high value. For 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.

Wiegand, E., V. Balasubramanian and D. W. Smith (1989). "Immunity to tuberculosis from the perspective of pathogenesis." *Infect Immun* 57: 3671-3676.

Thoen, C. O. (1988). "Tuberculosis." *J. Am. Vet. Med. Assoc* 193(9): 1045-1048.

Arora, B. M. (1986). Tuberculosis in wildlife in India. Summer Institute on Health, Production and Management in Wildlife, Indian Veterinary Institute.

Snider, D. E., Jr., W. D. Jones and R. C. Good (1984). "The usefulness of phage typing *Mycobacterium tuberculosis* isolates." *Am. Rev. Respir. Dis* 130: 1095-1099.

Mycobacteriophage typing of *Mycobacterium tuberculosis* isolates was used as an epidemiologic aid in investigating the transmission of tuberculosis in community, industrial, and institutional outbreaks. The technique was also useful in other situations, e.g., documenting congenital transmission of infection and distinguishing exogenous reinfection from endogenous reactivation. Additional studies are indicated to further explore the value of phage typing for tracking the transmission of tuberculosis in the community

Wallach, J. D. and W. J. Boever (1983). Tuberculosis. *Diseases of Exotic Animals.*: 791-792.

Saunders, G. (1983). "Pulmonary *Mycobacterium tuberculosis* infection in a circus elephant." *J. Am. Vet. Med. Assoc* 183(11): 1311-1312.

Devine, J. E., W. J. Boever and E. Miller (1983). "Isoniazid therapy in an Asiatic elephant (*Elephas maximus*)." *Journal of Zoo and Wildlife Medicine* 14: 130-133.

Woodford, M. H. (1982). "Tuberculosis in wildlife in the Ruwenzori National Park, Uganda (Part II)." *Trop. Anim. Hlth. Prod* 14(3): 155-160.

The results of post-mortem examinations of 90 warthog (*Phacochoerus aethiopicus*) conducted in the Ruwenzori National Park, Uganda during a survey of tuberculous infection in wildlife are described. Nine per 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 harbour 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.

Jones, W. D., Jr. and R. C. Good (1982). "Hazel elephant redux (letter)." *Am. Rev. Respir. Dis* 125(2): 270.

Full text. A recent letter from Greenberg, Jung and Gutter reported the untimely death of Hazel Elephant with *Mycobacterium tuberculosis* infection. The authors concluded that the animal trainer, who was found to have cavitary tuberculosis, was probably the source of infection. The conclusion was based on data available at the time. The isolates from Hazel Elephant and the animal trainer were submitted to us for further study the state health departments of Louisiana and Florida. Using the methodology and classification scheme previously described, we found that the cultures were of different phage types. The isolate from the elephant was type A0 (7), and the isolate from the trainer was type A1 (7,13,14). The isolates differed by lysis with one major phage (MTPH 5) and two auxiliary phages (MTPH 13 and 14). We have previously used phage typing of *M. tuberculosis* in several well-defined outbreaks as an adjunct to other epidemiologic procedures. The isolates were typed without the laboratory's knowing epidemiologic relationships between cases. The results indicated that *M. tuberculosis* transmitted from one individual to another retained the same phage-type characteristics. In the present study, our phage-type results suggest that the animal trainer and the elephant were infected from two different sources and that occurrence of disease in the animal and the trainer was coincidental. We are still evaluating phage typing as a procedure for use in tuberculosis epidemiology and can accept selected cultures for phage typing in special situations if we are contacted before the cultures are submitted.

Thoen, C. O. and E. M. Himes (1981). *Tuberculosis. Infectious diseases of wild mammals*. J. W. Davis, L. H. Karstad and D. O. Trainer. Ames, Iowa, The University of Iowa Press.

Mann, P. C., M. Bush, D. L. Janssen, E. S. Frank and R. J. Montali (1981). "Clinicopathologic correlations of tuberculosis in large zoo mammals." *J. Am. Vet. Med. Assoc* 179(11): 1123-1129.

In August 1978, a black rhinoceros at the National Zoological Park died with generalized tuberculosis caused by *Mycobacterium bovis*. A 2nd black rhinoceros was euthanatized 9 months after *M bovis* was cultured from its lungs. After these 2 deaths, numerous large zoo mammals that had been potentially exposed were subjected to various procedures to ascertain their status regarding tuberculosis. The procedures were: intradermal tuberculin testing, evaluation of delayed hypersensitivity reaction on biopsy specimens, enzyme-linked immunosorbent assay (ELISA) testing,

and culture of various secretions and organs. Several of the animals in this series died during the study. These were necropsied and examined for evidence of mycobacterial infection. The results of tuberculin testing varied from species to species and from site to site within a species. Delayed hypersensitivity responses generally correlated well with the amount of swelling at the tuberculin site. In some cases, however, positive reactions were found without any delayed hypersensitivity response. Results of ELISA testing were confirmatory in tuberculous animals. Several species were judged to be nonspecific reactors, based on positive or suspect tuberculin test results, with negative ELISA results and necropsy findings.

Gutter, A. (1981). Mycobacterium tuberculosis in an Asian elephant. Proc.Am.Assoc.Zoo Vet.

Greenberg, H. B., R. C. Jung and A. E. Gutter (1981). "Hazel Elephant is dead (of tuberculosis) (letter)." Am. Rev. Respir. Dis 124(3): 341.

Full text. Hazel Elephant was only 35 years old (by our estimate) when she died. She was cooperative and trusting to the last. Had we known about her illness sooner, we could have saved her. The Mycobacterium tuberculosis, var hominis that killed Hazel was sensitive to our drugs at the following levels: INH to 0.2mcg/ml; PAS to 2 mcg/ml; R to 1 mcg/ml; and MAB to 5 mcg/ml. Hazel worked and performed for a travelling circus. Ordinarily good-humored and loving, she had been off her feed for weeks. She became listless and apathetic, her eyes lost their sparkle, and she lacked her customary elan. Nonetheless, Hazel continued to perform for the children and do her other chores until she came to New Orleans. When Hazel got to New Orleans, she could barely move. The circus's bosses called for help. They brought her to the hospital at the Audubon Park and Zoological Garden. As soon as we saw Hazel, we admitted her to the isolation ward. We have her fluids, electrolytes, and antibiotics. We got cultures and other clinical laboratory tests. We comforted Hazel and tried to put her at ease. It was too late. She fell to the ground, her rheumy eyes gazed at us pitifully, her respirations failed, and she died. Hazel's postmortem examination took six hours. She was an emaciated Asian elephant whose lungs were filled with caseating granulomata. Since microscopy showed myriads of acid-fast bacilli, we examined everyone who had, or who thought they had, contact with Hazel. We found three persons with positive tuberculin skin test results. None had tuberculous disease. Fortunately, Hazel had been kept away from other animals. Hazel's circus did not wait for the results of our autopsy. It left Louisiana. The U.S. Public Health Service tracked it down and found the man, an animal trainer with cavitary tuberculosis, who probably gave Hazel her fatal disease. Now another health department will have to deal with the circus and its animals.

Thoen, C. O., K. Mills and M. P. Hopkins (1980). "Enzyme linked protein A: An enzyme-linked immunosorbent assay reagent for detecting antibodies in tuberculous exotic animals." Am. J. Vet. Res 41(5): 833-835.

An enzyme-linked immunosorbent assay (ELISA) was developed, using protein A labeled with horseradish peroxidase for detecting antibodies in tuberculous exotic animals (llamas, rhinoceroses, elephants). The modified ELISA provides a rapid procedure for screening several animal species simultaneously for tuberculosis without the production of specific anti-species conjugates. Heat-killed cells of Mycobacterium bovis and M. avium and purified protein-derivative tuberculin of M. bovis were used as antigens for ELISA.

Thoen, C. O. and E. M. Himes (1980). Mycobacterial infections in exotic animals. The comparative pathology of zoo animals. R. J. Montali and G. Migaki. Washington, D.C., Smithsonian Institution Press: 241-245.

Mycobacteria were isolated from 59% of the 826 specimens submitted from exotic animals suspected of having tuberculosis. *Mycobacterium bovis* and *Mycobacterium tuberculosis* accounted for 61% of the isolations from nonhuman primates. *Mycobacterium bovis* was the organism most frequently isolated from hoofed animals and *Mycobacterium avium* was most commonly isolated from birds. The distribution, pathogenesis, diagnosis, and control of tuberculosis in exotic animals is discussed.

Chandrasekharan, K. (1979). Common diseases of elephants. State Level Workshop on Elephants, College of Veterinary and Animal Sciences, Kerala Agricultural University.

Thoen, C. O., W. D. Richards and J. L. Jarnagin (1977). "Mycobacteria isolated from exotic animals." *J. Am. Vet. Med. Assoc* 170(9): 987-990.

von Benten, K., H. H. Fiedler, U. Schmidt, L. C. Schultz, G. Hahn and L. Dittrich (1975). "Occurrence of tuberculosis in zoo mammals; a critical evaluation of autopsy material from 1970 to the beginning of 1974." *Deutsche Tierärztliche Wochenschrift* 82(8): 316-318.

Pinto, M. R. M., M. R. Jainudeen and R. G. Panabokke (1973). "Tuberculosis in a domesticated Asiatic elephant *Elephas maximus*." *Vet. Rec* 93(26): 662-664.

A case of tuberculosis in a domesticated Asiatic elephant, *Elephas maximus*, was diagnosed on post-mortem examination. The causal organism was identified as *Mycobacterium tuberculosis* var *hominis* on the basis of cultural, biochemical and virulence studies. Microscopically, the lesions resembled tuberculous lesions as seen in man and other domestic animals, but an important difference was the apparent absence of Langerhan's type giant cells. The problems associated with the clinical diagnosis of tuberculosis in the elephant are discussed.

Gorovitz, C. (1969). "Tuberculosis in an African elephant." *Am. Assoc. Zoo Vet. Newsletter* January 20.

Seneviratna, P., S. G. Wettimuny and D. Seneviratna (1966). "Fatal tuberculosis pneumonia in an elephant." *VM SAC* 60: 129-132.

A fatal case of tuberculosis pneumonia with anemia and helminthiasis in a Ceylon elephant is reported. Acid-fast organisms resembling *Mycobacterium tuberculosis* and tubercular nodules were seen in large numbers in sections of the lung.

Gorovitz, C. (1962). "Tuberculosis in an African elephant." *Nord Vet Med* 14(Supl 1): 351-352.

Selye, H. (1956). Recent progress in stress research, with reference to tuberculosis. Personality, stress, and tuberculosis. P. J. Sparer. New York, Int. Univ. Press: 45-64.

Holmes, T. H. (1956). Multidiscipline studies of tuberculosis. Personality, stress, and tuberculosis. P. J. Sparer. New York, Int. Univ. Press: 65-125.

Halloran, P. O. (1955). "A bibliography of references to diseases in wild mammals and birds." *Am. J. Vet. Res* 16(part 2): 161.

Curasson, G. (1942). *Traite de pathologie exotique veterinaire et comparee*. Paris, Vigot Freres.

- Griffith, A. S. (1939). "Infections of wild animals with tubercle and other acid-fast bacilli." *Proc. R. Soc. Med* 32: 1405-1412.
- Winogradradsky, S. (1938). "La microbiologie ecologique ses principes - son procede." *Ann. Inst. Pasteur* 64(6): 715-730.
- Urbain, A. (1938). Tuberculosis in wild animals in captivity. *Ann. Inst. Pasteur Tuberculose chez animaux sauvages en captivite.* 61: 705-730.
- Iyer, A. K. (1937). "Veterinary science in India, ancient and modern with special reference to tuberculosis." *Agric. Livest. India* 7: 718-724.
- Curasson, G. (1936). *Treatise on the pathology of exotic animals.* Paris, Vigot Freres,.
- Datta, S. C. A. (1934). "Report of the pathology section." *Ann. Rep. Imp. Inst. Vet. Research Muktesar:* 25-33.
- Baldrey, F. S. H. (1930). "Tuberculosis in an elephant." *J. R. Army Vet. Corp* 1: 252.
- Bopayya, A. B. (1928). "Tuberculosis in an elephant." *Indian Veterinary Journal* 5: 142-145.
- Narayanan, R. S. (1925). "A case of tuberculosis in an elephant." *J. Comp. Pathol* 38: 96-97.
- Ishigami, T. (1918). "The influence of psychic acts on the progress of pulmonary tuberculosis." *Am. Rev. Tuberc* 2: 470-484.
- Thieringer, H. (1911). About tuberculosis in an elephant. *Berl. Tierarztl. Wschr Ueber Tuberkulose bei einem Elefanten.* 27: 234-235.
- Damman and Stedefeder (1909). Tuberculosis diseases in elephants with human type mycobacterium. *Deutsche Tierarztliche Wochenschrift Tuberkulose erkankung elefanten hervorgerufen durch Bazillen des sogenannten typus humanus.* 17: 345.
- Garrod, A. H. (1875). "Report on the Indian elephant which died in the society's gardens on July 7th, 1875." *Proc. Zool. Soc. Lond* 1875: 542-543.