Elephant Tuberculosis Publications (by Date)

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Songthammanuphap, S., et al. (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 (eIFNγ) 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.

Peters, H., et al. (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

Goosen, W. J., et al. (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., et al. (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.

Rosen, L. E., et al. (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., et al. (2019). "Human TB threat to wild elephants." Nature **571**(7764): 174.

Paudel, S., et al. (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., et al. (2019). "Tuberculosis threat in Asian elephants." Science **363**(6425): 356.

Miller, M. A., et al. (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., et al. (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., et al. (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 MTBCin greater detail.

Kerr, T. J., et al. (2019). "Seroprevalence of Mycobacterium tuberculosis Complex in Free-ranging African Elephants (Loxodonta africana) in Kruger National Park, South Africa." J Wildl Dis.

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 efore 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.

Yoshida, S., et al. (2018). "Mycobacterium caprae Infection in Captive Borneo Elephant, Japan." Emerg Infect Dis **24**(10): 1937-1940.

In 2016, disseminated tuberculosis caused by Mycobacterium caprae was diagnosed in a captive Borneo elephant in Japan. The bacterium was initially identified from clinical isolates. An isolate collected during a relapse showed isoniazid monoresistance and a codon 315 katG mutation.

Yano, T., et al. (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., et al. (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., et al. (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 Mycobaterium 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 κ = 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.

Rosen, L. E., et al. (2018). "Tuberculosis serosurveillance and management practices of captive African elephants (Loxodonta africana) in the Kavango-Zambezi Transfrontier Conservation Area." Transbound Emerg Dis **65**(2): e344-e354.

Transfrontier conservation areas represent an international effort to encourage conservation and sustainable development. Their success faces a number of challenges, including disease management in wildlife, livestock and humans. Tuberculosis (TB) affects humans and a multitude of non-human animal species and is of particular concern in sub-Saharan Africa. The Kavango-Zambezi Transfrontier Conservation Area encompasses five countries, including Zimbabwe, and is home to the largest contiguous population of free-ranging elephants in Africa. Elephants are known to be susceptible to TB; thus, understanding TB status, exposure and transmission risks to and from elephants in this area is of interest for both conservation and human health. To assess risk factors for TB seroprevalence, a questionnaire was used to collect data regarding elephant management at four ecotourism facilities offering elephant-back tourist rides in the Victoria Falls area of Zimbabwe. Thirty-five working African elephants were screened for Mycobacterium tuberculosis complex antibodies using the ElephantTB Stat-Pak and the DPP VetTB Assay for elephants. Six of 35 elephants (17.1%) were seropositive. The risk factor most important for seropositive status was time in captivity. This is the first study to assess TB seroprevalence and risk factors in working African elephants in their home range. Our findings will provide a foundation to develop guidelines to protect the health of captive and free-ranging elephants in the southern

African context, as well as elephant handlers through simple interventions. Minimizing exposure through shared feed with other wildlife, routine TB testing of elephant handlers and regular serological screening of elephants are recommended as preventive measures.

Paudel, S., et al. (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 occassion 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., et al. (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.

Lyashchenko, K. P., et al. (2018). "Spectrum of antibody profiles in tuberculous elephants, cervids, and cattle." Vet Microbiol **214**: 89-92.

Using multi-antigen print immunoassay and DPP((R)) VetTB Assay approved in the United States for testing captive cervids and elephants, we analyzed antibody recognition of MPB83 and CFP10/ESAT-6 antigens in Asian elephants (Elephas maximus) infected with Mycobacterium tuberculosis and in white-tailed deer (Odocoileus virginianus), fallow deer (Dama dama), elk (Cervus elaphus), and cattle (Bos taurus) infected with Mycobacterium bovis. Serum IgG reactivity to MPB83 was found in the vast majority of tuberculous cattle and cervid species among which white-tailed deer and elk also showed significant CFP10/ESAT-6 recognition rates with added serodiagnostic value. In contrast, the infected elephants developed antibody responses mainly to CFP10/ESAT-6 with MPB83 reactivity being relatively low. The findings demonstrate distinct patterns of predominant antigen recognition by different animal hosts in tuberculosis.

Hermes, R., et al. (2018). "Bronchoalveolar lavage for diagnosis of tuberculosis infection in elephants." Epidemiol Infect **146**(4): 481-488.

Tuberculosis (TB) has been known to affect elephants for thousands of years. It was put into spotlight when few circus elephants were diagnosed carrying Mycobacterium (M.) tuberculosis. Because of the zoonotic risk and high susceptibility to M. tuberculosis, periodic testing was enacted since, in captive breeding programmes. Presently, trunk wash is the recommended diagnostic

procedure for TB. Trunk wash, however, puts the operator at risk, has low sensitivity, and is prone to contamination. Here, bronchoalveolar lavage is described for the first time for TB diagnosis in elephants. Bronchial, trunk and mouth fluids were investigated using bacterial culture, M. tuberculosis complex (MTC)-specific real-time quantitative PCR (qPCR) and mycobacterial genus-specific qPCR for overall presence of mycobacteria or mycobacterial DNA including bacteria or DNA of closely related genera, respectively, in 14 elephants. Neither bacteria of the MTC nor their DNA were identified in any of the elephants. Yet, 25% of the cultures grew non-tuberculous mycobacteria (NTM) or closely related bacterial species. Furthermore, 85% of the samples contained DNA of NTM or closely related bacterial genera. This finding might explain continued false-positive results from various serological tests. From a zoonotic point of view, bronchoalveolar lavage is safer for the testing personal, has higher probability of capturing MTC and, through PCR, identifies DNA NTM in elephants. Yet, necessary endoscopic equipment, animal sedation and access to a TB reference laboratory might pose challenging requirements in remote conditions in some elephant range countries.

Barandongo, Z. R., et al. (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.

Zachariah, A., et al. (2017). "Mycobacterium tuberculosis in Wild Asian Elephants, Southern India." Emerg Infect Dis **23**(3): 504-506.

We tested 3 wild Asian elephants (Elephas maximus) in southern India and confirmed infection in 3 animals with Mycobacterium tuberculosis, an obligate human pathogen, by PCR and genetic sequencing. Our results indicate that tuberculosis may be spilling over from humans (reverse zoonosis) and emerging in wild elephants.

Veerasami, M., et al. (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

Simpson, G., et al. (2017). "Mycobacterium tuberculosis Infection among Asian Elephants in Captivity." Emerg Infect Dis **23**(3): 513-516.

Although awareness of tuberculosis among captive elephants is increasing, antituberculosis therapy for these animals is not standardized. We describe Mycobacterium tuberculosis transmission between captive elephants based on whole genome analysis and report a successful combination treatment. Infection control protocols and careful monitoring of treatment of captive elephants with tuberculosis are warranted.

Magnuson, R. J., et al. (2017). "Rapid screening for Mycobacterium tuberculosis complex in clinical elephant trunk wash samples." Res Vet Sci **112**: 52-58.

Mycobacterium tuberculosis can infect and be transmitted between elephants and humans. In elephants, the 'gold standard' reference test for detection of tuberculosis is culture, which takes a minimum of eight weeks for results and has limited sensitivity. A screening test that is rapid, easily implemented, and accurate is needed to aid in diagnosis of tuberculosis in elephants. Ninety-nine clinical trunk wash samples obtained from 33 elephants were utilized to validate three molecular extraction techniques followed by a polymerase chain reaction for detection of M. tuberculosis. Diagnostic sensitivity and specificity were estimated compared to culture. Kappa coefficients were determined between molecular results and various culture categories and serological test results. An internal amplification control was developed and assessed to monitor for PCR inhibition. One molecular test (the Column method) outperformed the other two, with diagnostic sensitivity and kappa agreement estimates of 100% (CI 57-100) and 0.46 (CI 0.2-0.74), respectively, compared to culture alone. The percentage of molecular-positive/culture-negative samples was 8.4% overall. The molecular extraction technique followed by PCR provides a much-needed rapid screening tool for detection of tuberculosis in elephants. Immediate procedures can be implemented to further assess PCR-positive animals and provide personnel biosecurity. While a positive result is not a definitive test for elephant tuberculosis, the molecular test results can be used to support current diagnostic procedures applied by veterinarians for treatment decisions to prevent the spread of tuberculosis in elephants.

Ghielmetti, G., et al. (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.

Chandranaik, B. M., et al. (2017). "Mycobacterium tuberculosis Infection in Free-Roaming Wild Asian Elephant." Emerg Infect Dis **23**(3): 555-557.

Postmortem examination of a wild Asian elephant at Rajiv Gandhi National Park, India, revealed nodular lesions, granulomas with central caseation, and acid-fast bacilli in the lungs. PCR and nucleotide sequencing confirmed the presence of Mycobacterium tuberculosis. This study indicates that wild elephants can harbor M. tuberculosis that can become fatal.

Casadevall, A. (2017). "Antibodies to mycobacterium tuberculosis." New England Journal of Medicine **376**(3): 283-285.

Burke, S. M., et al. (2017). "DETECTION OF AEROSOLIZED BACTERIA IN EXPIRED AIR SAMPLES FROM ASIAN ELEPHANTS (ELEPHAS MAXIMUS)." J Zoo Wildl Med **48**(2): 431-439.

Elephant-mediated transmission of tuberculosis is assumed to be similar to human models, which state close and prolonged contact with an infected individual is required for transmission. Although considered a risk factor for infection, several case studies have reported that close contact with an elephant is not always necessary for transmission, and the role of aerosolized bacteria remains unclear. To investigate aerosol-mediated transmission of pathogenic bacteria from elephants, a method for the detection of aerosols using an adapted sampling system was developed. A commensal bacterium was isolated from the upper respiratory tract of elephants (Elephas maximus) and was used as a proxy organism to detect aerosolized droplets in the sampling system. It was found that elephants are capable of producing aerosolized bacterial particles of a size small enough to remain airborne for prolonged periods and penetrate the lower regions of the human respiratory tract.

Zlot, A., et al. (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., et al. (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., et al. (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.

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Thapa, J., et al. (2016). "Mycobacterium orygis-Associated Tuberculosis in Free-Ranging Rhinoceros, Nepal, 2015." Emerg Infect Dis **22**(3): 570-572.

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 euthanatized 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., et al. (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 aceteate/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.

Paudel, S., et al. (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.

Hildebrandt, B., et al. (2016). Bronchalveloar 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 detomedine 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.

Vogelnest, L., et al. (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., et al. (2015). "First confirmed case of fatal tuberculosis in a wild Sri Lankan elephant." Gajah **41**: 28-31.

Niemeier, R. T., et al. (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., et al. (2015). "Mycobacterial Infections in Elephants." Tuberculosis, Leprosy and Mycobacterial Diseases of Man and Animals: The Many Hosts of Mycobacteria: 259-276.

Mikota, S. K., et al. (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.

Maslow, J. N. and S. K. Mikota (2015). "Tuberculosis in elephants-a reemergent disease: diagnostic dilemmas, the natural history of infection, and new immunological tools." Vet Pathol **52**(3): 437-440.

Tuberculosis (TB) in elephants has been described since ancient times. However, it was not until 1996 when infection with Mycobacterium tuberculosis was identified in a herd of circus elephants that significant research into this disease began. The epidemiology and natural history of TB were unknown in elephants since there had been no comprehensive screening programs, and diagnostic techniques developed for cervidae and bovidae were of unknown value. And, while precepts of test and slaughter were the norm for cattle and deer, this was considered untenable for an endangered species. With no precedent for the treatment of TB in animals, treatment regimens for elephants were extrapolated from human protocols, which guided changes to the Guidelines for the Control of Tuberculosis in Elephants. In the absence of diagnostic testing to confirm cure in elephants, the efficacy of these treatment regimens is only beginning to be understood as treated elephants die and are examined postmortem. However, because of pressures arising from public relations related to elephant husbandry and the added considerations of TB infection in animals (whether real or imagined), sharing of information to aid in research and treatment has been problematic. Here we review the challenges and successes of the diagnosis of tuberculosis in elephants and discuss the natural history of the disease to put the work of Landolfi et al on the immunological response to tuberculosis in elephants in perspective.

Lassausaie, J., et al. (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.

Landolfi, J. A., et al. (2015). "Pulmonary tuberculosis in Asian elephants (Elephas maximus): histologic lesions with correlation to local immune responses." Vet Pathol **52**(3): 535-542.

Although Mycobacterium tuberculosis infection is an important health concern for Asian elephants (Elephas maximus), no studies have evaluated the associated local immune responses or histologic lesions. In primates including humans, latent tuberculosis is distinguished by well-organized granulomas with TH1 cytokine expression, whereas active disease is characterized by poorly organized inflammation and local imbalance in TH1/TH2 cytokines. This study examined archival, formalin-fixed, paraffin-embedded lung samples from 5 tuberculosis-negative and 9 tuberculosis-positive Asian elephants. Lesions were assessed by light microscopy, and lymphoid infiltrates were characterized by CD3 and CD20 immunolabeling. Expression of TH1 (interferon [IFN]-gamma, tumor necrosis factor [TNF]-alpha) and TH2 (interleukin [IL]-4, IL-10, transforming growth factor

[TGF]-beta) cytokines was determined using in situ hybridization. In 6 of 9 samples, inflammation was similar to the pattern of primate active disease with low to moderate numbers of lymphocytes, most of which were CD20 positive. In 1 sample, inflammation was most similar to latent tuberculosis in primates with numerous CD3-positive lymphocytes. Expression of IFN-gamma was detected in 3 of 8 tuberculosis-positive samples. Expression of TNF-alpha was detected in 3 of 8 positive samples, including the one with latent morphology. Low-level expression of IL-4 was present in 4 of 8 positive samples. Only single positive samples displayed expression of IL-10 and TGF-beta. Tuberculosis-negative samples generally lacked cytokine expression. Results showed heterogeneity in lesions of elephant tuberculosis similar to those of latent and active disease in primates, with variable expression of both TH1 and TH2 cytokines.

Egelund, E. F., et al. (2015). "Population pharmacokinetics of rifampin in the treatment of Mycobacterium tuberculosis in Asian elephants." Journal of Veterinary Pharmacology and Therapeutics **38**(2): 137-143.

The objective of this study was to develop a population pharmacokinetic model for rifampin in elephants. Rifampin concentration data from three sources were pooled to provide a total of 233 oral concentrations from 37 Asian elephants. The population pharmacokinetic models were created using Monolix (version 4.2). Simulations were conducted using ModelRisk. We examined the influence of age, food, sex, and weight as model covariates. We further optimized the dosing of rifampin based upon simulations using the population pharmacokinetic model. Rifampin pharmacokinetics were best described by a one-compartment open model including first-order absorption with a lag time and first-order elimination. Body weight was a significant covariate for volume of distribution, and food intake was a significant covariate for lag time. The median Cmax of 6.07 μg/mL was below the target range of 8-24 μg/mL. Monte Carlo simulations predicted the highest treatable MIC of 0.25 μg/mL with the current initial dosing recommendation of 10 mg/kg, based upon a previously published target AUC0-24/MIC > 271 (fAUC > 41). Simulations from the population model indicate that the current dose of 10 mg/kg may be adequate for MICs up to 0.25 μg/mL. While the targeted AUC/MIC may be adequate for most MICs, the median Cmax for all elephants is below the human and elephant targeted ranges.

Chan, K. G., et al. (2015). "Multiphasic strain differentiation of atypical mycobacteria from elephant trunk wash." PeerJ **3**.

Background. Two non-tuberculous mycobacterial strains, UM_3 and UM_11, were isolated from the trunk wash of captive elephants in Malaysia. As they appeared to be identical phenotypes, they were investigated further by conventional and whole genome sequence-based methods of strain differentiation. Methods. Multiphasic investigations on the isolates included species identification with hsp65 PCR-sequencing, conventional biochemical tests, rapid biochemical profiling using API strips and the Biolog Phenotype Microarray analysis, protein profiling with liquid chromatography-mass spectrometry, repetitive sequence-based PCR typing and whole genome sequencing followed by phylogenomic analyses. Results. The isolates were shown to be possibly novel slow-growing schotochromogens with highly similar biological and genotypic characteristics. Both strains have a genome size of 5.2 Mbp, G+C content of 68.8%, one rRNA operon and 52 tRNAs each. They qualified for classification into the same species with their average nucleotide identity of 99.98% and tetranucleotide correlation coefficient of 0.99999. At the subspecies level, both strains showed 98.8% band similarity in the Diversilab automated repetitive sequence-based PCR typing system, 96.2% similarity in protein profiles obtained by liquid chromatography mass spectrometry, and a genomic distance that is close to zero in the phylogenomic tree constructed with conserved orthologs. Detailed epidemiological tracking revealed that the elephants shared a common habitat eight years apart, thus, strengthening the possibility of a clonal relationship between the two strains.

Waters, W. R., et al. (2014). "Relevance of bovine tuberculosis research to the understanding of human disease: historical perspectives, approaches, and immunologic mechanisms." Vet Immunol Immunopathol **159**(3-4): 113-132.

Pioneer studies on infectious disease and immunology by Jenner, Pasteur, Koch, Von Behring, Nocard, Roux, and Ehrlich forged a path for the dual-purpose with dual benefit approach, demonstrating a profound relevance of veterinary studies for biomedical applications. Tuberculosis

(TB), primarily due to Mycobacterium tuberculosis in humans and Mycobacterium bovis in cattle, is an exemplary model for the demonstration of this concept. Early studies with cattle were instrumental in the development of the use of Koch's tuberculin as an in vivo measure of cell-mediated immunity for diagnostic purposes. Calmette and Guerin demonstrated the efficacy of an attenuated M. bovis strain (BCG) in cattle prior to use of this vaccine in humans. The interferon-gamma release assay, now widely used for TB diagnosis in humans, was developed circa 1990 for use in the Australian bovine TB eradication program. More recently, M. bovis infection and vaccine efficacy studies with cattle have demonstrated a correlation of vaccine-elicited T cell central memory (TCM) responses to vaccine efficacy, correlation of specific antibody to mycobacterial burden and lesion severity, and detection of antigen-specific IL-17 responses to vaccination and infection. Additionally, positive prognostic indicators of bovine TB vaccine efficacy (i.e., responses measured after infection) include: reduced antigen-specific IFN-gamma, iNOS, IL-4, and MIP1-alpha responses; reduced antigen-specific expansion of CD4(+) T cells; and a diminished activation profile on T cells within antigen stimulated cultures. Delayed type hypersensitivity and IFN-gamma responses correlate with infection but do not necessarily correlate with lesion severity whereas antibody responses generally correlate with lesion severity. Recently, serologic tests have emerged for the detection of tuberculous animals, particularly elephants, captive cervids, and camelids. B cell aggregates are consistently detected within tuberculous lesions of humans, cattle, mice and various other species, suggesting a role for B cells in the immunopathogenesis of TB. Comparative immunology studies including partnerships of researchers with veterinary and medical perspectives will continue to provide mutual benefit to TB research in both man and animals.

Paudel, S., et al. (2014). "Molecular characterization of Mycobacterium tuberculosis isolates from elephants of Nepal." Tuberculosis (Edinb) **94**(3): 287-292.

Mycobacterium tuberculosis was cultured from the lung tissues of 3 captive elephants in Nepal that died with extensive lung lesions. Spoligotyping, TbD1 detection and multi-locus variable number of tandem repeat analysis (MLVA) results suggested 3 isolates belonged to a specific lineage of Indo-Oceanic clade, EAI5 SIT 138. One of the elephant isolates had a new synonymous single nucleotide polymorphism (SNP) T231C in the gyrA sequence, and the same SNP was also found in human isolates in Nepal. MLVA results and transfer history of the elephants suggested that 2 of them might be infected with M. tuberculosis from the same source. These findings indicated the source of M. tuberculosis infection of those elephants were local residents, presumably their handlers. Further investigation including detailed genotyping of elephant and human isolates is needed to clarify the infection route and eventually prevent the transmission of tuberculosis to susceptible hosts.

McGee, J. L., et al. (2014). "Prenatal passive transfer of mycobacterium tuberculosis antibodies in asian elephant (Elephas maximus) calves." Journal of Zoo and Wildlife Medicine **45**(4): 955-957.

Asian elephant (Elephas maximus) dams and their newborn calves were tested for Mycobacterium tuberculosis antibodies in serum. Blood was drawn from dams prior to calving and from calves on their day of birth. All six calves born to tuberculosis-reactive dams were also tuberculosis reactive, suggesting prenatal passive placental transfer of tuberculosis antibodies. In contrast, all three calves born to tuberculosis-nonreactive dams lacked detectable tuberculosis antibodies in pre-suckling or day-of-birth blood samples. Of the living tuberculosis-reactive calves observed from 1 to 11 yr of age, none exhibited clinical signs of tuberculosis infection or became tuberculosis culture positive. This is the first report of prenatal passive placental transfer of tuberculosis antibodies in elephants and demonstrates that detectible tuberculosis antibodies in newborn elephant calves should not be assumed to correlate with clinical tuberculosis.

Lassausaiae, J., et al. (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.

Landolfi, J. A., et al. (2014). "Differences in immune cell function between tuberculosis positive and negative Asian elephants." Tuberculosis (Edinb) **94**(4): 374-382.

Tuberculosis is an important health concern for Asian elephant (Elephas maximus) populations worldwide, however, mechanisms underlying susceptibility to Mycobacterium tuberculosis are unknown. Proliferative responses assessed via brominated uridine incorporation and cytokine expression measured by real-time RT-PCR were evaluated in peripheral blood mononuclear cell (PBMC) cultures from 8 tuberculosis negative and 8 positive Asian elephants. Cultures were stimulated with Mycobacterium bovis purified protein derivative (PPD-B), M. tuberculosis culture filtrate protein (CFP)-10, and Mycobacterium avium PPD (PPD-A). Following stimulation with PPD-B, proliferation was higher (alpha = 0.005) in positive samples; no significant differences were detected following CFP-10 or PPD-A stimulation. Tumor necrosis factor (TNF)-alpha, interleukin (IL)-12, and interferon (IFN)-gamma expression was greater in samples from positive elephants following stimulation with PPD-B (alpha = 0.025) and CFP-10 (alpha = 0.025 TNF-alpha and IL-12; alpha = 0.005 IFN-gamma). Stimulation with PPD-A also produced enhanced IL-12 expression in positive samples (alpha = 0.025). Findings suggested that differences in immune cell function exist between tuberculosis positive and negative elephants. Proliferative responses and expression of TNF-alpha, IL-12, and IFN-gamma in response to stimulation with PPD-B and CFP-10 differ between tuberculosis positive and negative elephants, suggesting these parameters may be important to tuberculosis immunopathogenesis in this species.

Hlokwe, T. M., et al. (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.

Fagen, A., et al. (2014). "Positive reinforcement training for a trunk wash in Nepal's working elephants: demonstrating alternatives to traditional elephant training techniques." J Appl Anim Welf Sci **17**(2): 83-97.

Many trainers of animals in the zoo now rely on positive reinforcement training to teach animals to voluntarily participate in husbandry and veterinary procedures in an effort to improve behavioral reliability, captive management, and welfare. However, captive elephant handlers in Nepal still rely heavily on punishment- and aversion-based methods. The aim of this project was to determine the effectiveness of secondary positive reinforcement (SPR) in training free-contact elephants in Nepal to voluntarily participate in a trunk wash for the purpose of tuberculosis testing. Five female elephants, 4 juveniles and 1 adult, were enrolled in the project. Data were collected in the form of minutes of training, number of offers made for each training task, and success rate for each task in performance tests. Four out of 5 elephants, all juveniles, successfully learned the trunk wash in 35 sessions or fewer, with each session lasting a mean duration of 12 min. The elephants' performance improved from a mean success rate of 39.0% to 89.3% during the course of the training. This study proves that it is feasible to efficiently train juvenile, free-contact, traditionally trained elephants in Nepal to voluntarily and reliably participate in a trunk wash using only SPR techniques.

van Sandwyk, J. H., et al. (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.

Stephens, N., et al. (2013). "Transmission of Mycobacterium tuberculosis from an Asian elephant (Elephas maximus) to a chimpanzee (Pan troglodytes) and humans in an Australian zoo." Epidemiol Infect **141**(7): 1488-1497.

Mycobacterium tuberculosis is primarily a pathogen of humans. Infections have been reported in animal species and it is emerging as a significant disease of elephants in the care of humans. With the close association between humans and animals, transmission can occur. In November 2010, a clinically healthy Asian elephant in an Australian zoo was found to be shedding M. tuberculosis; in September 2011, a sick chimpanzee at the same zoo was diagnosed with tuberculosis caused by an indistinguishable strain of M. tuberculosis. Investigations included staff and animal screening. Four staff had tuberculin skin test conversions associated with spending at least 10 hours within the elephant enclosure; none had disease. Six chimpanzees had suspected infection. A pathway of transmission between the animals could not be confirmed. Tuberculosis in an elephant can be transmissible to people in close contact and to other animals more remotely. The mechanism for transmission from elephants requires further investigation.

Schaftenaar, W., et al. (2013). "Retrospective serological investigation of bovine tuberculosis in two gemsbok (oryx gazelle gazelle) and an onager (equus hemionus onager)." Journal of Zoo and Wildlife Medicine **44**(4): 1036-1042.

In 1997 a 26-yr-old gemsbok (Oryx gazelle gazelle) died of bovine tuberculosis in a zoo. Three remaining gemsbok were administered the comparative tuberculin skin test repeatedly over a period of 5 mo. Two animals showed inconclusive results on the second test. All three gemsbok

were euthanatized. Mycobacterium bovis was isolated from one of those with an inconclusive skin test result, whereas Mycobacterium fortuitum was detected in the other gemsbok. Eight years later, an onager (Equus hemionus onager) died of bovine tuberculosis. This animal had been kept in the same building as the gemsbok. Three herd mates were culled after administering the comparative tuberculin skin test. They were all nonreactors and produced no evidence of tuberculosis at postmortem examination. Retrospectively, using plasma samples collected from the gemsbok and onagers, three antibody tests, Elephant TB STAT-PAK, multiantigen print immunoassay (MAPIA), and dual-path platform (DPP) VetTB (Chembio Diagnostic Systems Inc., Medford, New York, 11763, USA), were used to assess their diagnostic value for these species. The M. bovis-infected gemsbok tested strongly positive by Elephant TB STAT-PAK at the time of euthanasia and 5 mo earlier when the skin test was negative. This animal was not antibody reactive in MAPIA and DPP VetTB. No M. bovis-specific antibody was detected in the other two gemsboks by any of the immunoassays. Among the onagers, Elephant TB STAT-PAK, MAPIA, and DPP VetTB revealed gradually increasing antibody response in the animal that died of bovine tuberculosis, but not in the three disease-free herd mates euthanatized. Seroconversion in the M. bovis-infected onager was first noticed 5 yr before death when the tuberculin skin test was negative. Copyright 2013 by American Association of Zoo Veterinarians.

Ong, B. L., et al. (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., et al. (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 anthropozoonotic 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., et al. (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.a 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: aChembio Diagnostic Systems, Inc, Medford, NY, USA 11763.

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2006. Elephant tuberculosis diagnosis: implications for elephant management in Asian range countries. Proc. Am. Assoc. Zoo Vet. Annual. Conf. Pp. 142-143.
4. Obanda, V., J. Poghon, M. Yongo, I. Mulei, M. Ngotho, K. Waititu, J. Makumi, F. Gakuya, P. Omondi, R.C. Soriguer, and S. Alasaad. 2013. First report of fatal tuberculosis in a wild African elephant with past human-wildlife contact. Epidemiol. Infect. Pp. 1-5.

Maas, M., et al. (2013). "Facts and dilemmas in diagnosis of tuberculosis in wildlife." Comparative Immunology, Microbiology and Infectious Diseases **36**(3): 269-285.

Mycobacterium bovis, causing bovine tuberculosis (BTB), has been recognized as a global threat at the wildlife-livestock-human interface, a clear "One Health" issue. Several wildlife species have been identified as maintenance hosts. Spillover of infection from these species to livestock or other wildlife species may have economic and conservation implications and infection of humans causes public health concerns, especially in developing countries. Most BTB management strategies rely on BTB testing, which can be performed for a range of purposes, from disease surveillance to diagnosing individual infected animals. New diagnostic assays are being developed for selected wildlife species. This review investigates the most frequent objectives and associated requirements for testing wildlife for tuberculosis at the level of individual animals as well as small and large populations. By aligning those with the available (immunological) ante mortem diagnostic assays, the practical challenges and limitations wildlife managers and researchers are currently faced with are highlighted. © 2012 Elsevier Ltd.

Ghodbane, R. and M. Drancourt (2013). "Non-human sources of Mycobacterium tuberculosis." Tuberculosis (Edinb) **93**(6): 589-595.

Mycobacterium tuberculosis is a successful pathogen responsible for the vast majority of deadly tuberculosis cases in humans. It rests in a dormant form in contaminated people who constitute the reservoir with airborne interhuman transmission during pulmonary tuberculosis. M. tuberculosis is therefore regarded majoritary as a human pathogen. Here, we review the evidence for anthroponotic M. tuberculosis infection in non-human primates, other mammals and psittacines. Some infected animals may be sources for zoonotic tuberculosis caused by M. tuberculosis, with wild life trade and zoos being amplifying factors. Moreover, living animals and cadavers can scatter M. tuberculosis in the environment where it could survive for extended periods of time in soil where amoebae could play a role. Although marginal in the epidemiology of human tuberculosis, these data indicate that M. tuberculosis is not uniquely adapted to humans.

Feldman, M., et al. (2013). "Point prevalence and incidence of Mycobacterium tuberculosis complex in captive elephants in the United States of America." Veterinary Quarterly **33**: 25-29.

Comas, I., et al. (2013). "Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans." Nat Genet **45**(10): 1176-1182.

Tuberculosis caused 20% of all human deaths in the Western world between the seventeenth and nineteenth centuries and remains a cause of high mortality in developing countries. In analogy to other crowd diseases, the origin of human tuberculosis has been associated with the Neolithic Demographic Transition, but recent studies point to a much earlier origin. We analyzed the whole genomes of 259 M. Tuberculosis complex (MTBC) strains and used this data set to characterize global diversity and to reconstruct the evolutionary history of this pathogen. Coalescent analyses indicate that MTBC emerged about 70,000 years ago, accompanied migrations of anatomically modern humans out of Africa and expanded as a consequence of increases in human population density during the Neolithic period. This long coevolutionary history is consistent with MTBC displaying characteristics indicative of adaptation to both low and high host densities. © 2013 Nature America, Inc. All rights reserved.

Angkawanish, T., et al. (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., et al. (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., et al. (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.

Gagneux, S. (2012). "Host-pathogen coevolution in human tuberculosis." Philosophical Transactions of the Royal Society B: Biological Sciences **367**(1590): 850-859.

Tuberculosis (TB) is a disease of antiquity. Yet TB today still causes more adult deaths than any other single infectious disease. Recent studies show that contrary to the common view postulating an animal origin for TB, Mycobacterium tuberculosis complex (MTBC), the causative agent of TB, emerged as a human pathogen in Africa and colonized the world accompanying the Out-of- Africa migrations of modern humans. More recently, evolutionarily 'modern' lineages of MTBC expanded as a consequence of the global human population increase, and spread throughout the world following waves of exploration, trade and conquest. While epidemiological data suggest that the different phylogenetic lineages of MTBC might have adapted to different human populations, overall, the phylogenetically 'modern' MTBC lineages are more successful in terms of their geographical spread compared with the 'ancient' lineages. Interestingly, the global success of 'modern' MTBC correlates with a hypo-inflammatory phenotype in macrophages, possibly reflecting higher virulence, and a shorter latency in humans. Finally, various human genetic variants have been associated with different MTBC lineages, suggesting an interaction between human genetic

diversity and MTBC variation. In summary, the biology and the epidemiology of human TB have been shaped by the long-standing association between MTBC and its human host.

Yong, H., et al. (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., et al. (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.

Simons, S., et al. (2011). "Nontuberculous mycobacteria in respiratory tract infections, Eastern Asia." Emerging Infectious Diseases **17**(3): 343-349.

To characterize the distribution of nontuberculous mycobacteria (NTM) species isolated from pulmonary samples from persons in Asia and their association with pulmonary infections, we reviewed the literature. Mycobacterium avium complex bacteria were most frequently isolated (13%-81%) and were the most common cause of pulmonary NTM disease (43%-81%). Also pathogenic were rapidly growing mycobacteria (M. chelonae, M. fortuitum, M. abscessus). Among all NTM isolated from pulmonary samples, 31% (582/1,744) were considered clinically relevant according to American Thoracic Society diagnostic criteria. Most patients were male (79%) and had a history of tuberculosis (37%). In Asia, high prevalence of rapidly growing mycobacteria and a history of tuberculosis are distinct characteristics of pulmonary NTM disease. This geographic variation is not well reflected in the American Thoracic Society criteria for NTM infections and could be incorporated in future guidelines.

Orloski, K. (2011). Epidemiology of Tuberculosis in Elephants. Tuberculosis in Elephants: Science, Myth and Beyond, USDA, APHIS Center for Animal Welfare.

Murphree, R., et al. (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.

McManamon, R. and S. Terrell (2011). Elephant Postmortem Examination. Tuberculosis in Elephants: Science, Myth and Beyond, USDA, APHIS Center for Animal Welfare.

Kay, M. K., et al. (2011). "Evaluation of DNA extraction techniques for detecting Mycobacterium tuberculosis complex organisms in Asian elephant trunk wash samples." J Clin Microbiol **49**(2): 618-623.

Rapid and sensitive diagnostic assays for the detection of tuberculous mycobacteria in elephants are lacking. DNA extraction with PCR analysis is useful for tuberculosis screening in many species but has not been validated on elephant trunk wash samples. We estimated the analytical sensitivity and specificity of three DNA extraction methods to detect Mycobacterium tuberculosis complex organisms in trunk wash specimens. A ZR soil microbe DNA kit (ZR) and a traditional salt and ethanol precipitation (TSEP) approach were evaluated under three different treatment conditions: heat treatment, phenol treatment, and contamination with Mycobacterium avium. A third approach, using a column filtration method, was evaluated for samples contaminated with soil. Trunk wash samples from uninfected elephants were spiked with various concentrations of M. bovis cells and subjected to the described treatment conditions prior to DNA extraction. Extracted DNA was amplified using IS6110-targeted PCR analysis. The ZR and TSEP methods detected as low as 1 to 5 M. bovis cells and 10 M. bovis cells, respectively, per 1.5 ml of trunk wash under all three conditions. Depending on the amount of soil present, the column filtration method detected as low as 5 to 50 M. bovis cells per 1.5 ml of trunk wash. Analytical specificity was assessed by DNA extraction from species of nontuberculous mycobacteria and amplification using the same PCR technique. Only M. bovis DNA was amplified, indicating 100% analytical specificity of this PCR technique. Our results indicate that these DNA extraction techniques offer promise as useful tests for detection of M. tuberculosis complex organisms in elephant trunk wash specimens.

Dumonceaux, G. A., et al. (2011). "Genitourinary and pulmonary multidrug resistant Mycobacterium tuberculosis infection in an Asian elephant (Elephas maximus)." J Zoo Wildl Med **42**(4): 709-712.

A female Asian elephant (Elephas maximus) developed vaginal and trunk discharge. Cultures were positive for pan-susceptible Mycobacterium tuberculosis. Isoniazid and pyrazinamide were given rectally and monitored by serum levels. After being trained at 10 mo to accept oral dosing, treatment was changed and rifampin was added. Oral medications were administered for another 10 mo. A year after completion of therapy, the vaginal discharge increased and cultures yielded M. tuberculosis, resistant to isoniazid and rifampin. Treatment with oral ethambutol, pyrazinamide, and enrofloxacin and intramuscular amikacin was initiated. Although followup cultures became negative, adverse reactions to medications precluded treatment completion. Due to public health concerns related to multidrug resistant M. tuberculosis (MDR-TB), the elephant was euthanized. Postmortem smears from the lung, peribronchial, and abdominal lymph nodes yielded acid-fast bacteria, although cultures were negative. This case highlights important considerations in the treatment of M. tuberculosis in animals and the need for a consistent approach to diagnosis, treatment, and follow-up.

Stanton, J. J., et al. (2010). "Detection of pathogenic elephant endotheliotropic herpesvirus in routine trunk washes from healthy adult Asian elephants (Elephas maximus) by use of a real-time quantitative polymerase chain reaction assay." Am J Vet Res **71**(8): 925-933.

OBJECTIVE: To investigate the pathogenesis and transmission of elephant endotheliotropic herpesvirus (EEHV1) by analyzing various elephant fluid samples with a novel EEHV1-specific real-time PCR assay. ANIMALS: 5 apparently healthy captive Asian elephants (Elephas maximus) from the same herd. PROCEDURES: A real-time PCR assay was developed that specifically detects EEHV1. The assay was used to evaluate paired whole blood and trunk-wash samples obtained from the 5 elephants during a 15-week period. Deoxyribonucleic acid sequencing and viral gene subtyping analysis were performed on trunk-wash DNA preparations that had positive results for EEHV1. Viral gene subtypes were compared with those associated with past fatal cases of herpesvirus-associated disease within the herd. RESULTS: The PCR assay detected viral DNA to a level of 1,200 copies/mL of whole blood. It was used to detect EEHV1 in trunk secretions of 3 of the 5 elephants surveyed during the 15-week period. Viral gene subtyping analysis identified 2 distinct elephant herpesviruses, 1 of which was identical to the virus associated with a previous fatal case of herpesvirus-associated disease within the herd. CONCLUSIONS AND CLINICAL RELEVANCE: EEHV1 was shed in the trunk secretions of healthy Asian elephants. Trunk secretions may provide a mode of transmission for this virus. Results of this study may be useful for the diagnosis, treatment, and management of EEHV1-associated disease and the overall management of captive elephant populations.

Sang, R., et al. (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., et al. (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 thefundamental 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., et al. (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)-c; tumor necrosis factor (TNF)-a; and transforming growth factor (TGF)-b were measured using elephant-specific, real-time reverse transcriptionpolymerase 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-a and lower levels of TGF-b than seronegative animals; these differences between groups were statistically significant when levels were analyzed as categorical variables. Trends toward higher levels of IFN-c 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-a 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 intothe immunopathogenesis of elephant tuberculosis.

Angkawanish, T., et al. (2010). "Mycobacterium tuberculosis infection of domesticated Asian elephants, Thailand." Emerg Infect Dis **16**(12): 1949-1951.

Alexander, K. A., et al. (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.

(2010). Guidelines for the control of tuberculosis in elephants 2010. Proceedings of 114th Annual Meeting of the United States Animal Health Association. **114:** 578-639.

Steingart, K. R., et al. (2009). "Performance of purified antigens for serodiagnosis of pulmonary tuberculosis: A meta-analysis." Clinical and Vaccine Immunology **16**(2): 260-276.

Serological antibody detection tests for tuberculosis may offer the potential to improve diagnosis. Recent metaanalyses have shown that commercially available tests have variable accuracies and a limited clinical role. We reviewed the immunodiagnostic potential of antigens evaluated in research laboratories (in-house) for the serodi-agnosis of pulmonary tuberculosis and conducted a meta-analysis to evaluate the performance of comparable antigens. Selection criteria included the participation of at least 25 pulmonary tuberculosis patients and the use of purified antigens. Studies evaluating 38 kDa, MPT51, malate synthase, culture filtrate protein 10, TbF6, antigen 85B, αa-crystallin, 2,3-diacyltrehalose, 2,3,6-triacyltrehalose, 2,3,6,6'-tetraacyltrehalose 2'-sulfate, cord factor, and TbF6 plus DPEP (multiple antigen) were included in the meta-analysis. The results demonstrated that (i) in sputum smear-positive patients, sensitivities significantly ≥50% were provided for recombinant malate synthase (73%; 95% confidence interval [CI], 58 to 85) and TbF6 plus DPEP (75%; 95% CI, 50 to 91); (ii) protein antigens achieved high specificities; (iii) among the lipid antigens, cord factor had the best overall performance (sensitivity, 69% [95% CI, 28 to 94]; specificity, 91% [95% CI, 78 to 97]); (iv) compared with the sensitivities achieved with single antigens (median sensitivity, 53%; range, 2% to 100%), multiple antigens yielded higher

sensitivities (median sensitivity, 76%; range, 16% to 96%); (v) in human immunodeficiency virus (HIV)-infected patients who are sputum smear positive, antibodies to several single and multiple antigens were detected; and (vi) data on seroreactivity to antigens in sputum smear-negative or pediatric patients were insufficient. Potential candidate antigens for an antibody detection test for pulmonary tuberculosis in HIV-infected and -uninfected patients have been identified, although no antigen achieves sufficient sensitivity to replace sputum smear microscopy. Combinations of select antigens provide higher sensitivities than single antigens. The use of a case-control design with healthy controls for the majority of studies was a limitation of the review. Efforts are needed to improve the methodological quality of tuberculosis diagnostic studies.

Mikota, S. K., et al. (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.
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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 Publci Policy, Cummings School of Veterinary Medicine, Tufts University**:** 74-84.

Michel, A. L., et al. (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., et al. (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

Esple, I. W., et al. (2009). "Pulmonary infection due to mycobacterium bovis in a black rhinoceros (Diceros bicomis minor) in South Africa." Journal of Wildlife Diseases **45**(4): 1187-1193.

We report a case of tuberculosis due to infection with Mycobacterium bovis in an elderly male black rhinoceros (Diceros bicornis minor) from the Limpopo Province in South Africa. The animal was euthanized due to very poor condition, old age, and dental attrition. Necropsy examination revealed two small nonencapsulated granulomas (~40-mm diameter) in the dorsocaudal lobe of the left lung. Sequencing of isolated crude lung tissue PCR product and boiled lung culture samples confirmed that the causative organism was M. bovis. Genotyping revealed limited similarities with M. bovis strains isolated thus far from South African cattle or wildlife. The source of the infection could not be determined. This case illustrates that M. bovis could impact conservation of free-ranging rare and endangered species. Effective diagnostics are urgently needed for different animal species, such as white or black rhinoceroses, to certify with a reasonable degree of certainty that these animals are free of tuberculosis in natural habitats. © Wildlife Disease Association 2009.

Duncan, A. E., et al. (2009). "Application of elephant TB stat-pak assay and mapia (Multi-Antigen Print Immunoassay) for detection of tuberculosis and monitoring of treatment in black rhinoceros (Diceros bicornis)." Journal of Zoo and Wildlife Medicine **40**(4): 781-785.

Many wildlife species including rhinos are susceptible to infection with Mycobacterium tuberculosis or M. bovis. Antemortem diagnostic testing in large exotic hoof stock species has been limited by challenges associated with test administration, sample collection, and interpretation. Hence, a simple, rapid, blood-based test is needed. Two confirmed M. tuberculosis-infected black rhinoceros and one exposed suspect were evaluated for antibody responses using a lateral-flow rapid test (ElephantTB STAT-PAK) and multi-antigen print immunoassay (MAPIA). All three animals were seropositive by both tests. MAPIA detected antibodies to ESAT-6, CFP10, and MPB83 antigens. When the rhinos were treated with antitubercular therapeutics, their antibody responses gradually declined. One rhinoceros died approximately 9 mo after initiation of treatment and showed an increase in antibody titer shortly before death. The other two rhinoceros, which were treated for 1 and 2 yr, respectively, had no clinical signs or positive culture for M. tuberculosis at the time of necropsy performed 2 or 6 yr later for unrelated reasons. The antibody levels in these rhinos continued to be significantly decreased. The findings suggest that the ElephantTB STAT-PAK and MAPIA may be useful tools to detect M. tuberculosis infection and monitor treatment in black rhinoceros. © 2009 American Association of Zoo Veterinarians.

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.

Meyers, D. A., et al. (2008). Evaluation of acute phase proteins for diagnosis of inflammation in Asian elephants (Elephas maximus). Proc American Associaton of Zoo Veterinarians and Assoc of Reptile and Amphibian Veterinarians.

In many domestic species, routine hematology assays are useful diagnostic tools to diagnose inflammatory conditions. Unlike other species, these hematologic tests apparently are insensitive indicators of inflammation in elephants.1 We studied a novel group of blood proteins, called acute phase proteins, which increase during inflammatory conditions, for their usefulness in diagnosing elephants with inflammatory diseases. Although these proteins currently are useful in humans and domestic animals, each species has a different set of important proteins that must be individually investigated.2 We tested several acute phase proteins (C-reactive protein, alpha-1 glycoprotein, alpha-1 antitrypsin, serum amyloid A, haptoglobin, fibrinogen, ceruloplasmin, and albumin) as well as complete blood counts, chemistry panels, serum protein electrophoresis, and 3-D gel electrophoresis to determine their usefulness for diagnosing different types of inflammatory conditions in Asian elephants (*Elephas maximus*). Animals with inflammatory conditions were classified as those individuals with known illnesses such as mycobacteriosis, arthritis, nail bed abscesses, and malignant tumors. Control animals were thoseanimals that were suspected to not have any inflammation and be healthy at the time of testing as determined by physical examination and obtaining a thorough medical history.

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

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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.

Sreekumar, E., et al. (2007). "Molecular characterization and expression of Interferon-γ of Asian elephant (Elephas maximus)." Veterinary Immunology and Immunopathology **118**(1-2): 75-83.

Tuberculosis (TB) caused by Mycobacterial organisms has emerged as one of the major diseases in captive elephants. In vitro Interferon-gamma (IFN-γ) assay is being used as an ancillary test for early detection of TB in domestic and captive wild animals. In the present study, basic sequence information and immunological cross-reactivity of this major cytokine of Asian elephants were

explored. At predicted amino acid level, IFN-γ of Asian elephant showed maximum identity to that of horse (73%). Other IFN-γ amino acid sequences that showed high level identity were that of giant panda (72%), dog (71%), nine-banded armadillo (69%), cattle (63%) and human (62%). IFN-γ promoter sequences of Asian elephant, human, cattle and mouse showed high level conservation of the putative transcription factor binding sites, TATA box and transcriptional start site. The functionally important human IFN-γ promoter elements, such as AP-2IRE-BE, YY1-γIFN-BED, ATFCS and AP-1γINF binding sites, were absolutely conserved in the corresponding elephant sequence. There was only a single nucleotide variation in the other two important elements, NFAT-γINF and IFN-γPE, indicating the highly conserved regulation of IFN-γ expression across different species. Phylogenetic analysis based on IFN-γ protein sequences revealed a closer relation of Asian elephants and nine-banded armadillo. This shows a closer evolution of these members of Afrotheria and Xenarthra, respectively; and supports the previous reports based on mitochondrial DNA studies. In Western blot analysis, IFN-γ of Asian elephant expressed in Escherichia coli was detected using an anti-bovine IFN-γ monoclonal antibody, indicating immunological cross-reactivity.

Renwick, A. R., et al. (2007). "Bovine tuberculosis in southern African wildlife: A multi-species host-pathogen system." Epidemiology and Infection **135**(4): 529-540.

This review examines the current situation of bovine tuberculosis (bTB) in southern African savannah systems, and uses theory on multi-species host-pathogen systems to suggest possible options for future research and management. In southern Africa, the buffalo (Syncerus caffer) and the Kafue lechwe [Marsh antelope] (Kobus leche) have been found to be maintenance hosts for this disease, but the importance of other host species is becoming apparent. The role of other host species in the maintenance and spread of the disease varies, depending on the spatial distribution and resource utilization patterns of the species, disease susceptibility, transmission modes and the ecology of both host(s) and vector(s). Future research needs to identify the pathogenicity of bTB in each of the host species, and the mechanisms and rates of inter- and intra-specific transmission among different species, in order to develop multi-host models to understand the development and spread of the disease.

Mikota, S. K., et al. (2007). Comparison of four serological tests and culture to determine tuberculosis infection in captive elephants in Nepal. Proceedings AAZV,AAWV,AZA/NAG Joint Conference.

Lacasse, C., et al. (2007). "Two cases of atypical mycobacteriosis caused by Mycobacterium szulgai associated with mortality in captive African elephants (Loxodonta africana)." J. Zoo. Wildl. Med **38**(1): 101-107.

Mycobacterium szulgai was associated with mortality in two captive African elephants (Loxodonta africana) housed at Lincoln Park Zoo. The first elephant presented with severe, acute lameness of the left rear limb. Despite extensive treatments, the animal collapsed and died 13 mo after initial presentation. Necropsy revealed osteomyelitis with loss of the femoral head and acetabulum and pulmonary granulomas with intralesional M. szulgai. The second elephant collapsed during transport to another institution with no premonitory clinical signs. This animal was euthanized because of prolonged recumbency. Granulomatous pneumonia with intralesional M. szulgai was found at necropsy. Two novel immunoassays performed on banked serum samples detected antibody responses to mycobacterial antigens in both infected elephants. It was not possible to determine when the infection was established or how the elephants were infected. When reviewing the epidemiology of this organism in humans, however, transmission between elephants seemed unlikely because human-to-human transmission of this organism has never been reported and a third elephant in the herd was not affected. In addition to Mycobacterium bovis and Mycobacterium tuberculosis, atypical mycobacterial organisms need to be considered potentially pathogenic in elephants.

Hamilton, K., et al. (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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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., et al. (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., et al. (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., et al. (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., et al. (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., et al. (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., et al. (2006). "Tuberculosis in elephants: antibody responses to defined antigens of Mycobacterium tuberculosis, potential for early diagnosis, and monitoring of treatment." Clin. Vaccine

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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., et al. (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., et al. (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

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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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Contraceptive vaccines (CV) may provide viable and valuable alternatives to the presently available methods of contraception. The molecules that are being explored for CV development either target gamete production [luteinizing hormone-releasing hormone (LHRH)/GnRH, FSH], gamete function [sperm antigens and oocyte zona pellucida (ZP)], and gamete outcome (HCG). CV targeting gamete production have shown varied degrees of efficacy; however, they either affect sex steroids causing impotency and/or show only a partial rather than a complete effect in inhibiting gametogenesis. However, vaccines based on LHRH/GnRH are being developed by several pharmaceutical companies as substitutes for castration of domestic pets, farm and wild animals, and for therapeutic anticancer purposes such as in prostatic hypertrophy and carcinoma. These vaccines may also find applications in clinical situations that require the inhibition of increased secretions of sex steroids, such as in uterine fibroids, polycystic ovary syndrome, endometriosis and precocious puberty. CV targeting molecules involved in gamete function such as sperm antigens and ZP proteins are exciting choices. Sperm constitute the most promising and exciting target for CV. Several sperm-specific antigens have been delineated in several laboratories and are being actively explored for CV development. Studies are focused on delineating appropriate sperm-specific epitopes, and increasing the immunogenicity (specifically in the local genital tract) and efficacy on the vaccines. Anti-sperm antibody (ASA)-mediated immunoinfertility provides a naturally occurring model to indicate how a vaccine might work in humans. Vaccines based on ZP proteins are quite efficacious in producing contraceptive effects, but may induce oophoritis, affecting sex steroids. They are being successfully tested to control feral populations of dogs, deer, horses and elephants, and populations of several species of zoo animals. The current research for human applicability is focused on delineating infertility-related epitopes (B-cell epitopes) from oophoritis-inducing epitopes (T-cell epitopes). Vaccines targeting gamete outcome primarily focus on the HCG molecule. The HCG vaccine is the first vaccine to undergo Phase I and II clinical trials in humans. Both efficacy and lack of immunopathology have been reasonably well demonstrated for this vaccine. At the present time, studies are focused on increasing the immunogenicity and efficacy of the birth control vaccine, and examining its clinical applications in various HCG-producing cancers. The present article will focus on the current status of the anti-sperm, anti-ZP, anti-LHRH/GnRH and anti-HCG vaccines

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We recently described the clinical presentation and treatment of 18 elephants from six herds infected with TB. Treatment protocols and methods varied between herds to include both oral and rectal dosing using multiple drug doses and formulations. In this paper we present information regarding the pharmacokinetics (PK) of isoniazid (INH) in elephants and provide suggestions regarding initial treatment regimens. Forty-one elephants received INH daily by either oral or rectal administration with different formulations. Population PK analysis was performed using Non-linear Mixed Effect Modeling (NONMEM). Results of oral administration indicated that compared with premixed INH solution, the drug exposure was highest with a suspension prepared freshly with INH powder. When INH was concomitantly given as an admixture over food, Tmax was delayed and variability in drug absorption was significantly increased. Compared with oral administration, similar drug exposures were found when INH was dosed rectally. The data generated suggest that a starting dose of 7.5 mg/kg of INH is appropriate for initial TB treatment in elephants when

premixed solution is administered directly into the oropharynx or rectal vault and 4 mg/kg are when INH is administered following immediate suspension from powdered form

Lyashchenko, K., et al. (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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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., et al. (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 "effectivelytreated" 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., et al. (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.

Ceylan, E., et al. (2005). "A new parameter in the detection of tuberculosis activity: Reactive oxygen metabolites." Respiration **72**(2): 156-159.

Background and Objectives: In countries with a high frequency of tuberculosis, there are problems not only with active lung tuberculosis but also with past lung tuberculosis. Cases with sequel tuberculosis very frequently present with complaints like tuberculosis, and it is very hard to determine whether it is a sequel tuberculosis complication or reactivation of tuberculosis. In this study, we measured the serum reactive oxygen metabolite (ROM) levels of patients with active pulmonary tuberculosis and healthy controls, and investigated if these metabolites can be used as a criterion for differentiation between active pulmonary tuberculosis and sequel pulmonary tuberculosis. Methods: 40 patients with active tuberculosis, 35 patients with sequel pulmonary tuberculosis and 30 healthy control subjects with a similar age range and sex distribution were included in the study. Serum total ROM levels were detected in the patients and control group. Results: Mean serum ROM values were 994 ± 236, 551 ± 135 and 236 ± 59 U/l among active lung tuberculosis cases, sequel lung tuberculosis cases and the healthy control group, respectively. As a result of these findings, serum ROM levels of active lung tuberculosis cases and sequel lung tuberculosis cases were significantly higher than those of the control group (both $p < 0.001$). The serum ROM levels of active lung tuberculosis cases were also significantly higher than those of sequel lung tuberculosis cases ($p < 0.001$). Conclusions: In the light of our findings, it may be assumed that serum total ROM values can be used as an activity criterion in the differentiation of active lung tuberculosis and sequel lung tuberculosis. Copyright © 2005 S. Karger AG.

Stringfield, C. E., et al. (2004). Epidemiologic investigation of a *Mycobacterium tuberculosi*s 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 rhinocerus (*Diceros bicornis*) was diagnosed with *Mycobacerium 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 reporte d 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.

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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., et al. (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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So African veterinarians, Mike Bester, Larry Killmar, Janet Payeur, ARC/OVI, Thomas Hildebrant, Eric Zeehandelar, Kevin Reily, Denise SoFranko.

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Ziccardi, M., et al. (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 infections[1]. 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 species[3]. 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., et al. (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., et al. (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., et al. (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.

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Turenne, C., et al. (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 1from an axillary lymph node.

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

Payeur, J. B., et al. (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., et al. (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.

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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 safe 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., et al. (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., et al. (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.

Ratanakorn, P. (2001). Elephant Health Problems and Management in Cambodia, Lao and Thailand. A Research Update on Elephants and Rhinos; Proceedings of the International Elephant and Rhino Research Symposium, Vienna, June 7-11, 2001, Schuling Verlag.

Montali, R. J., et al. (2001). "Mycobacterium tuberculosis in zoo and wildlife species." OIE Revue

Scientifique et Technique **20**(1): 291-303.

Tuberculosis caused by Mycobacterium tuberculosis and M. tuberculosis-like organisms has been identified in a wide range of species, including non-human primates, elephants and other exotic ungulates, carnivores, marine mammals and psittacine birds. Disease associated with M. tuberculosis has occurred mostly within captive settings and does not appear to occur naturally in free-living mammals. Mycobacterium tuberculosis probably originated as an infection of humans, but from the zoonotic standpoint, non-human primates, Asian elephants and psittacine birds have the potential to transmit this disease to humans. However, the overall prevalence of disease in these susceptible species is low and documented transmissions of M. tuberculosis between animals and humans are uncommon. Mycobacterium tuberculosis causes progressive pulmonary disease in mammals and a muco-cutaneous disease in parrots. In all cases, the disease can disseminate and be shed into the environment. Diagnosis in living animals is based on intradermal tuberculin testing in non-human primates, culturing trunk secretions in elephants, and biopsy and culture of external lesions in parrots. Ancillary testing with deoxyribonucleic acid probes and nucleic acid amplification, and enzyme-linked immunosorbent assays have been adapted to some of these species with promising results. Additionally, new guidelines for controlling tuberculosis in elephants in the United States of America, and programmes for tuberculosis prevention in animal handlers have been established.

Mikota, S. K., et al. (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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Ziccardi, M., et al. (2000). Tuberculosis in zoo ungulates:Survey results and surveillance plan. Proc. AAZV and IAAAM Joint Conf.

Shojaei, H., et al. (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., et al. (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 form 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.

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Larsen, R. S., et al. (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., et al. (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.

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

Mangold, B. J., et al. (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 administrating 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. Erkrg. 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., et al. (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 (Antilope 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., et al. (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 end points.

Michalak, K., et al. (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., et al. (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 cative 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., et al. (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 theses, 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 fowwoing 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., et al. (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 contained. The association of tuberculosis with malnutrition and poverty has long been recognized and the need to address these basic issues as as crucial as specific measures against the disease itself.

Dalovision, J. R., et al. (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., et al. (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., et al. (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 hypergic 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.

Wiegeshaus, E., et al. (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., et al. (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., et al. (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 by 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 amphibia 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 A_0 (7), and the isolate from the trainer was type A_1 (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 page 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., et al. (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., et al. (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. The 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., et al. (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 Sicences, Kerala Agricultural University.

Thoen, C. O., et al. (1977). "Mycobacteria isolated from exotic animals." J. Am. Vet. Med. Assoc **170**(9): 987-990.

von Benten, K., et al. (1975). "Occurrence of tuberculosis in zoo mammals; a critical evaluation of autopsy material from 1970 to the beginning of 1974." Deutsche Tierarztliche Wochenschrift **82**(8): 316-318.

Pinto, M. R. M., et al. (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., et al. (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.