Mycobacterium tuberculosis in zoo and wildlife species

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Summary

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.

Keywords

Animal diseases – Mycobacteria – Mycobacterium tuberculosis – Tuberculosis – Wildlife – Zoo animals – Zoonoses.

Introduction

Various forms of mycobacterial disease have plagued non-domestic animals since the beginnings of menageries and organised animal exhibits. The earlier literature reflects a high prevalence of tuberculosis from the mid-nineteenth until the mid-twentieth century, and records from some of the older zoos depict problems with this disease in exhibit animals in the United States of America (USA) and Europe (14, 17, 19, 47). The earliest reported observation of tuberculosis in a zoo dates from 1836; the infection in a chimpanzee in the London zoo was described by Owen (50). In fact, the highest incidences of tuberculosis were due to *Mycobacterium*

tuberculosis and *M. bovis* in zoo primates, with losses of up to 40% in some collections (57). The introduction of glass barriers between the public and animals in the 1930s decreased the incidence of tuberculosis in zoo primates (20, 59, 61).

In ungulates, particularly the larger land mammals, tuberculosis is caused by *M. bovis* in the majority of cases. However, tuberculosis due to *M. tuberculosis* has also occurred in elephants, rhinoceroses, tapirs, and some exotic bovine species from Africa. In addition, *M. tuberculosis* has been documented in some exotic carnivores and psittacine birds (33) and a *M. tuberculosis*-like disease has been

described in marine mammals (1). With the exception of a report of M. tuberculosis in a turtle (66), this human strain of mycobacteria has been limited to warm-blooded animals.

In the mid-1970s, M. tuberculosis was the third most frequently isolated mycobacterium (11%) among a total of 263 mycobacterial isolates obtained from zoos and wildlife parks in the USA (68). Tuberculosis in wildlife has been studied to a lesser degree, but with a few exceptions, infection does not appear to occur naturally in most free-living mammals and birds.

This paper will focus on the host range, epidemiology, diagnosis, clinical and pathological features, and control of M. tuberculosis in non-domestic species, with emphasis on the zoonotic aspects in elephants and non-human primates. As a known global re-emerging disease in human populations with a zoonotic potential, M. tuberculosis is of critical importance to wildlife veterinarians, public health officials and zoos.

Mycobacterium tuberculosis complex: nomenclature and characteristics

Classically, Mycobacterium tuberculosis complex has comprised four species of tubercle bacilli, namely: M. tuberculosis, M. bovis, M. africanum and M. microti (46) (it has been proposed that M. canetti, a smooth variant of M. tuberculosis, also be included in this group [52, 53]). Humans are the natural host and reservoir for M. tuberculosis, and domestic cattle the natural host and reservoir for M. bovis. Mycobacterium africanum is a human strain distributed in Africa, and M. microti is found in the meadow vole (Microtus agrestis), in which it causes a tuberculous disease (33). The term tuberculosis, by convention, is used to refer to diseases caused exclusively by M. tuberculosis complex organisms, of which M. tuberculosis and M. bovis are the most important. Infections caused by other mycobacterial species (sometimes called tuberculoid bacilli, atypical mycobacteria, non-tuberculous mycobacteria [NTM], or mycobacteria other than tuberculous mycobacteria [MOTT]) are referred to as mycobacterioses (25, 55, 68). However, the terms avian tuberculosis (caused by M. avium complex which includes M. intracellulare) and fish tuberculosis (caused by M. marinum and others) are frequently encountered in the veterinary literature (23, 28, 35).

Despite some morphological and biochemical differences, M. tuberculosis and M. bovis are fairly closely related. Disease produced by these organisms in humans, primates and hoofed mammals cannot be distinguished by morphological or histological examination of tissues alone (69). Recently, a special form of polymerase chain reaction (PCR), termed spoligotyping, has been adapted to formalin-fixed tissues to discern M. tuberculosis from other mycobacteria in histological sections of tuberculous tissues (74). In experimental infection studies (used previously as biological assays to distinguish among certain mycobacteria),

M. tuberculosis is highly pathogenic in guinea-pigs and non-human primates and of low pathogenicity in cattle and rabbits; M. bovis, in contrast, is highly virulent in the latter two species (10, 69).

Historical aspects of *Mycobacterium tuberculosis*

The presence of mycobacterial infections has been traced back to ancient times, with mention of osteoarcheological evidence of mycobacteria in 6,000-year-old mummies in Egypt (23). Mycobacterium tuberculosis may have developed through the domestication of cattle infected with M. bovis, the probable evolutionary precursor of M. tuberculosis, some 15 to 20 thousand years ago. The scourge of human tuberculosis is said to have commenced in Europe during the Middle Ages and spread to the New World in the 1600s (62, 63). During the 1800s and early 1900s, bovine tuberculosis was the most prevalent infectious disease of cattle and swine in the USA, causing more losses among farm animals than all other infectious diseases combined. The National Cooperative State-Federal Bovine Tuberculosis Eradication Program, was initiated in 1917 by the United States Department of Agriculture, and has brought the USA close to eradicating bovine tuberculosis. This programme is based on surveillance for tuberculosis at slaughter and subsequent investigation of herds epidemiologically connected to infected herds. Although M. bovis has a wide host range and can be infectious for humans, M. tuberculosis usually causes minor, non-progressive infections in domestic cattle (22). However, cattle inadvertently infected with M. tuberculosis by tuberculous owners can be sensitised to tuberculin and develop false-positive reactions for M. bovis infections (64).

Mycobacterium tuberculosis in zoo and wildlife species

Primates
Epidemiology
Simian tuberculosis caused by M. tuberculosis is an important and well-documented disease often occurring as laboratory colony or zoo epidemics with high morbidity and mortality (15, 18). Tuberculosis is probably non-existent in wild primates remote from human habitation. When infection occurs, the usual cause is human contact after capture in countries of Asia, particularly the Indian subcontinent. The incidence is therefore greatest in captive Old World primates, with a high prevalence in rhesus monkeys (Macaca mulatta), in which the disease spreads rapidly with death occurring in less than one year and usually within four to six months. Infections are commonly spread by infectious droplet nuclei, but also through cage-mate aggression and indirect contact with infected animals (by fomites or accidentally by management or medical procedures such as tattooing or gastric intubation). The cynomolgus monkey (Macaca

fascicularis) appears to be less susceptible, although this has been considered a relative and not an absolute resistance (29, 54).

In contrast to *M. bovis* infections, *M. tuberculosis* causes a more fulminant disease in most non-human primates; however, the *M. tuberculosis* organism is less virulent in baboons and great apes. In general, tuberculosis is less frequent in species originating in Africa and uncommon in species from South America. Exposure (e.g. in mixed exhibits) to infected primate species from other geographic areas can result in higher attack rates in some of these seemingly more resistant species (32, 39).

Clinical and pathological aspects

In the more common form of simian *M. tuberculosis* infection, specific clinical signs are minimal or absent until the disease is well-advanced. Animals may show progressive lethargy, anorexia and coughing, although the latter sign is common in primates with other respiratory conditions. Sometimes the animals may be simply found dead. Suppurative wounds with localised lymphadenopathy should always be regarded as a possible sign of mycobacterial infection.

At necropsy, tuberculous infections may be characterised by extensive pulmonary disease with caseation and cavitation and marked bronchial lymphadenopathy. Disseminated forms with a miliary pattern are common in fulminant tuberculosis in young macaques. Lesions in baboons, apes and New World primates are usually intermediate in severity between the pathological findings in rhesus monkeys and humans. Classically, the histological appearance of simian tuberculous lesions has been described as containing few or no Langhans' giant cells, with absence of calcification and fibrosis (57). However, McClure has noted all of these microscopic features in some chimpanzees and macaques with *M. tuberculosis* infections (36).

Diagnosis and control

The intradermal tuberculin test is the most frequently used ante-mortem assay for diagnosing tuberculosis in non-human primates. Antigens derived from either *M. tuberculosis* or *M. bovis* are apparently equally effective in eliciting a cross-reactive response in an animal infected with either organism (67). Most non-human primates show a low response to tuberculin and require 1,000 to 10,000 times the antigen concentration needed for humans. Old tuberculins fit this requirement and are the products recommended for use in primates (49, 73). One exception is the orang-utan (*Pongo pygmaeus*) which appears to have a greater sensitivity to tuberculins than monkeys and other ape species. This has resulted in a high prevalence of non-specific responders in orang-utans. Although uncommon, *M. tuberculosis* has been reported in captive orang-utans, therefore this species usually requires comparative tuberculin testing and more invasive diagnostic procedures to discount the presence of pathogenic mycobacteria (5).

For standard tuberculin testing in primates, the eyelid (palpebra) and periumbilical skin of the abdomen are the sites used for the tuberculin test, which is usually performed three to five times at two-week intervals during quarantine, and at six-month intervals thereafter. Animals with advanced tuberculosis, those exposed to measles, or animals receiving anti-tuberculosis drugs may be anergic, leading to a false-negative test result (49, 73). Cultures for mycobacteria may be obtained from throat swabs or gastric lavage. Radiography is less diagnostic because calcified granulomas, typical of tuberculosis in humans and other animals, do not usually occur in primates. More recently, deoxyribonucleic acid (DNA) probes and nucleic acid amplification procedures have been used to diagnose primate tuberculosis. Enzyme-linked immunosorbent assays (ELISA) have also been employed as an adjunct to detect exposed or diseased monkeys during colony outbreaks (8, 56). However, a standardised, validated ELISA for tuberculosis in non-human primates is not currently available.

Treatment of clinical cases of tuberculosis in primates has not generally been advocated in laboratory colonies, although in one epidemic involving rhesus monkeys and cynomolgus monkeys, streptomycin and isoniazid were used successfully in a significant number of animals (75). An experimental tuberculosis model has been recently developed in rhesus monkeys to evaluate diagnostic assays, therapeutic agents and vaccines for use in both human and non-human primates (4). Information on therapeutics in laboratory primates could be of use for endangered or genetically important zoo primates.

Currently, fewer primates are imported from the wild than previously, and importation and quarantine requirements are becoming more stringent. As a result, more primates are being propagated in research and zoo facilities, thus minimising the risk of introducing human (or bovine) tuberculosis into the colonies.

Elephants

Tuberculosis was first described in elephants over 2,000 years ago (27, 37). Despite statements that Asian elephants (*Elephas maximus*) may be susceptible to *M. bovis*, *M. tuberculosis* has been the organism detected in all elephant tuberculosis cases from which isolates were obtained. As in the other non-domestic species previously discussed, elephants are most likely to contract tuberculosis subsequent to contact with infected humans. Naturally-occurring tuberculosis has not been reported in free-living Asian elephants or African elephants (*Loxodonta africanum*). Tuberculosis occurs most frequently in Asian elephants (7, 21, 58). Of those cases in African elephants, only one recorded case was confirmed by culture (72). These differences may reflect a closer association of the Asian elephant with humans, rather than a true species susceptibility. In a retrospective medical study of 379 elephants, eight Asian elephants died of tuberculosis between 1908 and 1994 (41). This study included elephants in zoos, but excluded privately-owned animals, therefore the true

incidence is likely to be greater. Much of the following information is based on the recent emergence of *M. tuberculosis* in Asian elephants in the USA (42).

Epidemiology

The recent emergence of *M. tuberculosis* in elephants in North America began with the death of two travelling circus elephants in 1996 (3). Between August 1996 and 3 June 2000, seventeen cases of *M. tuberculosis* were confirmed in Asian elephants in North America (42). Elephants from eight herds in Illinois, California, Arkansas, Missouri and Florida were affected. Contact had previously occurred between elephants in five of the herds. *Mycobacterium tuberculosis* was isolated from twelve elephants *ante mortem* and from five elephants *post mortem*. Five distinct strains of *M. tuberculosis* were identified, based on restriction fragment length polymorphism (RFLP) of isolates from eleven elephants. From this study, the prevalence of *M. tuberculosis* was estimated to be approximately 3.0% in the captive elephant population in North America (42).

Clinical and pathological aspects

Signs attributed to tuberculosis in elephants are mostly non-specific, e.g. inappetance, weight loss, reluctance to do strenuous work and occasionally subcutaneous ventral oedema. More typically, premonitory ante-mortem signs are absent. The major pathological changes in elephants with *M. tuberculosis* occur primarily in the lungs and thoracic lymph nodes with lesser involvement of extra-thoracic sites. In the less extensive cases, firm granulomatous nodules, sometimes with caseous foci, are noted in the bronchial lymph nodes and pulmonary tissue (Fig. 1). Elephants with extensive involvement of both lungs usually die with severe caseo-calcareous and cavitating lesions (Fig. 2). These often result in large pulmonary abscesses from which
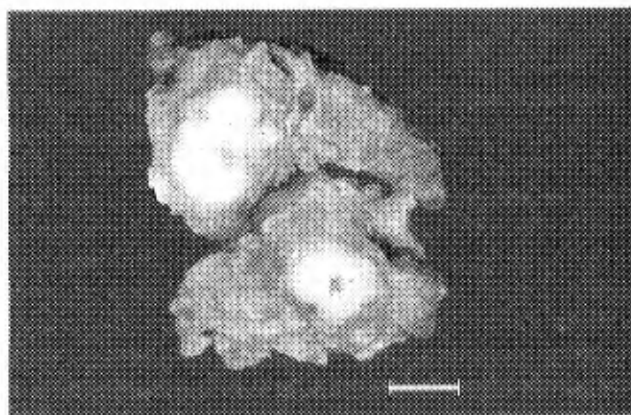
Fig. 1
Lung segments (after fixation in formalin) depicting an early case of tuberculosis with granulomas containing central caseation necrosis from a forty-five-year-old female Asian performing elephant from the United States of America
(Bar = 1 cm)
The larger nodules are surrounded by smaller satellite epithelioid granulomas. *Mycobacterium tuberculosis* was cultured from the lesions
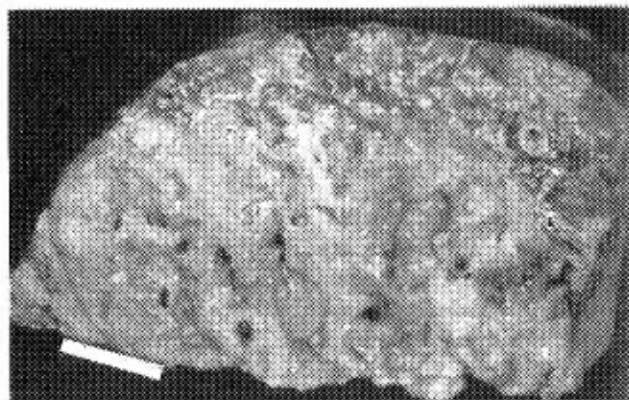
Fig. 2
Advanced caseo-calcareous tuberculous pneumonia
(Bar = 5 cm)
Segment of pulmonary lobe from an Asian elephant (index case) from the 1996 outbreak of *Mycobacterium tuberculosis* in the United States of America
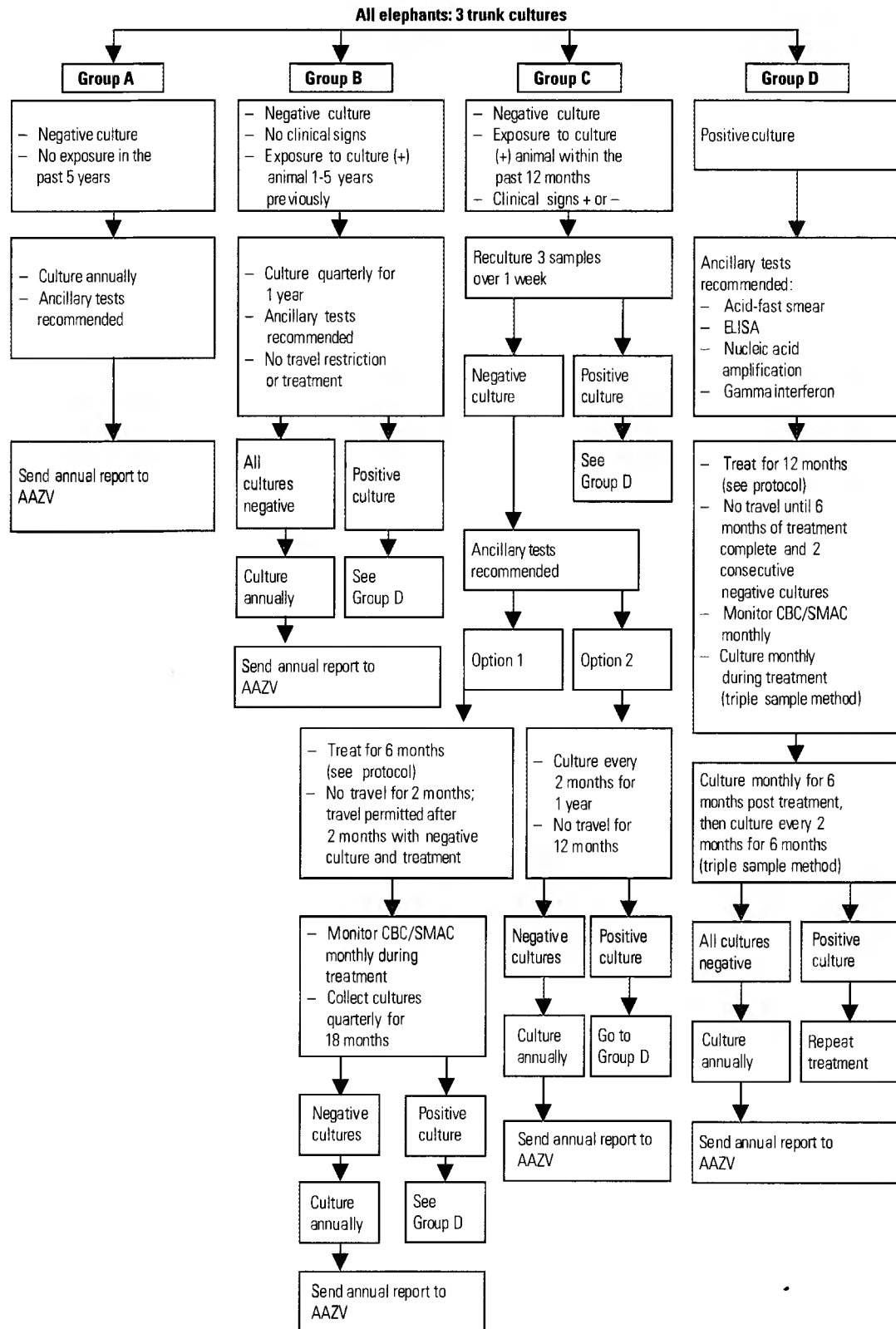Photo: courtesy of Dr Hailu Kinde, California Animal Health and Food Safety Laboratory, University of California, Davis

M. tuberculosis and opportunistic bacteria such as *Pseudomonas aeruginosa* have been isolated.

Characteristic histological findings include epithelioid granulomas with some giant cell formation in the initial lymph node and pulmonary lesions and extensive caseous and pyogranulomatous pneumonia in the advanced forms. Although sparsely distributed, acid-fast bacilli are more easily found in central areas of caseation in the lungs, rather than in the lymph nodes where they are typically rare. Bronchial and tracheal tuberculous plaques, and caseous and mucopurulent exudate in the nasal and trunk passages have been noted in both the early and late stages of tuberculosis, suggesting that the shedding of mycobacteria may occur at any stage of the disease.

Diagnosis and control

In 1997, a National Tuberculosis Working Group for Zoo and Wildlife Species in the USA formulated the *Guidelines for the Control of Tuberculosis in Elephants* (71) to specify criteria for the testing, surveillance and treatment of elephants for tuberculosis. Intradermal tuberculin tests to diagnose tuberculosis in elephants have correlated poorly with mycobacterial culture results, with high percentages of false-negatives in culture-positive animals. Currently, the test considered most reliable for diagnosis of tuberculosis in elephants is based on the culture of respiratory secretions obtained by trunk lavage (or 'washes'). Sterile saline is instilled into the nostrils and then recovered in a plastic bag. Three samples are collected on separate days (26). Elephant herds in the USA are tested by this method on an annual basis or more frequently if cases of tuberculosis are detected or if the herd is known to have been previously exposed to tuberculosis. The flow sheet in Figure 3 presents the testing schedule in detail. A

All elephants: 3 trunk cultures

Group A	Group B	Group C	Group D
– Negative culture – No exposure in the past 5 years	– Negative culture – No clinical signs – Exposure to culture (+) animal 1-5 years previously	– Negative culture – Exposure to culture (+) animal within the past 12 months – Clinical signs + or –	Positive culture

Group A:

– Culture annually
– Ancillary tests recommended

Send annual report to AAZV

Group B:

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

All cultures negative	Positive culture
Culture annually	See Group D

Send annual report to AAZV

Group C:

Reculture 3 samples over 1 week

Negative culture	Positive culture
	See Group D

Ancillary tests recommended

Option 1	Option 2

Option 1:

– Treat for 6 months (see protocol)
– No travel for 2 months; travel permitted after 2 months with negative culture and treatment

– Monitor CBC/SMAC monthly during treatment
– Collect cultures quarterly for 18 months

Negative cultures	Positive culture
Culture annually	See Group D

Send annual report to AAZV

Option 2:

– Culture every 2 months for 1 year
– No travel for 12 months

Negative cultures	Positive culture
Culture annually	Go to Group D

Send annual report to AAZV

Group D:

Ancillary tests recommended:
– Acid-fast smear
– ELISA
– Nucleic acid amplification
– Gamma interferon

– Treat for 12 months (see protocol)
– No travel until 6 months of treatment complete and 2 consecutive negative cultures
– Monitor CBC/SMAC monthly
– Culture monthly during treatment (triple sample method)

Culture monthly for 6 months post treatment, then culture every 2 months for 6 months (triple sample method)

All cultures negative	Positive culture
Culture annually	Repeat treatment

Send annual report to AAZV

AAZV : American Association of Zoo Veterinarians
CBC : complete blood count
ELISA : enzyme-linked immunosorbent assay
SMAC : serum chemistry profile

Fig. 3
Culture and ancillary tests for the control of tuberculosis in captive elephants in the United States of America (71)

nucleic acid amplification test which utilises transcription mediated amplification to specifically replicate ribosomal ribonucleic acid (rRNA) from bacteria of the M. tuberculosis complex, has been used on elephant trunk-wash samples as an ancillary test and has shown a moderate sensitivity and a high specificity for M. tuberculosis. In addition, a multiple-antigen ELISA was recently evaluated for serological detection of tuberculous animals in captive elephant herds which had M. tuberculosis culture-positive cases (31). Discriminant analysis was used to determine the combination of antigens that accurately predicted the true infection status in the greatest number of animals. Preliminary results suggest that this multiple-antigen ELISA may be an effective screening test for elephants, although further research is required.

Unlike the 'test and depopulate' procedures used for the National Cooperative State-Federal Bovine Tuberculosis Eradication Program in the USA, the Guidelines allow for the treatment and rehabilitation of tuberculous and suspect elephants under conditions of quarantine and travel restrictions (42, 71). Anti-tuberculosis drugs recently used in elephants include isoniazid, pyrazinamide, rifampin and ethambutol. The dose and treatment protocols have been published recently (42). These drugs have been administered to elephants rectally and by direct oral administration, and blood levels consistent with levels known to be therapeutically effective in humans have been achieved (13; S.K. Mikota, unpublished data). Results have been favourable, with culture-positive elephants reverting to culture-negative status shortly after the initiation of treatment. However, continued monitoring of these recent cases will be required to determine the efficacy of long-term treatment.

Other ungulates

As previously noted, M. tuberculosis infection in domestic cattle is usually a localised, non-progressive disease with minor lesions of the retropharyngeal and thoracic lymph nodes. Sheep and goats are also fairly resistant to infection. Although relatively infrequently, M. tuberculosis has occurred as pulmonary infections in Bovidae from Africa, including zoo-exhibited oryx (Oryx gazella beisa) and black buck (Antilope cervicapra). The source of the tuberculosis in the oryx was believed to be an overflow of a moat surrounding a colony of rhesus monkeys infected with M. tuberculosis (34, 51).

Extensive pulmonary tuberculosis from M. tuberculosis was identified in a fourteen-year-old male bongo (Tragelaphus eurycerus) housed in a zoo in Canada. Mycobacterium xenopi, but not M. tuberculosis, was recovered from the lymph node of the female bongo exhibit mate (K. Mehren, personal communication). Interestingly, M. tuberculosis was diagnosed in an elephant, a black rhinoceros (Diceros bicornis) and three mountain goats (Oreamnos americanus) at a zoo in the USA where both bongo had been housed ten years previously (C. Sedgwick, personal communication). However, the isolate from the bongo was demonstrated by RFLP analysis to be unrelated to these cases in the USA and the epidemiological origin of the bongo case remains unknown.

Earlier accounts have described M. tuberculosis infections with extensive pulmonary disease in a camel, a giraffe (Giraffa camelopardalis) and a black rhinoceros (33). Acid-fast organisms from pulmonary abscesses in a great Indian rhinoceros (Rhinoceros unicornis) in a zoo in India were attributed to M. tuberculosis (perhaps erroneously), based on morphology rather than isolation of the organism (30). Two cases of tuberculosis attributed to M. tuberculosis were reported in tapirs (Tapirus terrestris) from two separate zoos in Germany and Belgium (33).

Mycobacterium tuberculosis and Mycobacterium tuberculosis-like organisms in other mammals

Terrestrial carnivores

Mycobacterium tuberculosis infection has rarely been reported in exotic carnivores and case reports cited include disseminated tuberculosis in a European otter (Lutra lutra) and a polar bear (Ursus maritimus) from zoos in Europe (33).

Otarid seals

Tuberculosis-producing mycobacteria have been previously described in both captive and wild otarid seals (fur seals, Arctocephalus spp. and sea lions, Neophoca spp.) from Australia, New Zealand and Tasmania in the late 1980s and early 1990s (16, 70, 77). The mycobacteria isolated in most cases belonged to the M. tuberculosis complex but demonstrated genetic and biochemical differences when compared to M. tuberculosis and M. bovis (1).

Psittacine birds

In addition to M. avium, psittacines are susceptible to infection with M. tuberculosis (33). Early accounts describe losses of up to 33% in collections of Old World parrots, due to M. tuberculosis (24). A recent report cites cases of M. tuberculosis in four Amazon parrots with localised nodular lesions on the head and neck, and involving the eye, retrobulbar tissues and sinuses, nares and oral cavities (76). Systemic disease occurs with lesions in the lungs, liver, heart and other sites. Histologically, typical caseous granulomas with giant cells are noted with acid-fast bacilli in the M. tuberculosis lesions. Tuberculous humans are usually not identified as sources of the avian disease, but in one case of tuberculosis in a green-winged macaw (Ara chloropterus) in New York, the owners had culture-confirmed pulmonary tuberculosis three to four years before the diagnosis in the bird (76). Mycobacterium tuberculosis isolated from biopsied lesions from the head of the macaw were identified as a non-drug resistant strain and by RFLP analysis as a common pattern seen in New York city. The owners had close contact and often fed the bird mouth to beak.

The natural history and pathogenesis of psittacine tuberculosis caused by M. tuberculosis differ from that of disease caused by M. avium. The latter is primarily a disease of the digestive tract with dissemination to visceral organs, rarely exhibiting muco-cutaneous lesions. Caseous granulomas with giant cells occur in the parrot lesions in both forms, but myriad acid-fast bacilli are usually observed in M. avium infection, whereas M. tuberculosis is usually associated with lower numbers of organisms (45).

Zoonotic aspects of *Mycobacterium tuberculosis*

Potential sources

Although infection has declined in the USA (6), M. tuberculosis infects approximately one-third of the population of the world and kills three million patients each year, making it the single most important infectious cause of death world-wide (55, 78). The most important zoonotic sources of M. tuberculosis have been non-human primates. Animal caretakers and veterinarians working at primate research facilities and zoos have been at greatest risk. In the 1970s, the annual tuberculin conversion rate in people exposed to primates was approximately 70/10,000 compared with less than 3/10,000 in the general population (29).

Recently, M. tuberculosis has been documented as a zoonotic disease in Asian elephants in the USA (38, 40). Following the diagnosis of M. tuberculosis in a herd of elephants in Illinois (as described earlier), eleven of twenty-two elephant handlers had positive intradermal tuberculin responses. Eight of the eleven positive tests were indicative of prior exposure and three of recent exposure. One elephant handler was culture-positive for M. tuberculosis and the RFLP analysis matched that of an elephant from Illinois. The original source of infection in this and the seven other herds is unknown.

Other zoo megavertebrates have been implicated in infecting zoo employees with M. tuberculosis complex organisms. Seven of twenty-four keepers from a zoo in the south-east of the USA converted to positive intradermal tuberculin tests, presumably caused by aerosol transmission of infection from an affected white rhinoceros (Ceratotherium simum simum). In this case, the rhinoceros was culture-positive for M. bovis, which apparently spread to colobus monkeys in a nearby facility. None of the individual handlers were clinically ill and all received preventive therapy with isoniazid. However, M. tuberculosis has affected other species of rhinoceros, as noted above, and could conceivably be spread in a similar way. The risk of exposure to M. tuberculosis can be considered higher in zoo settings than in most other work environments (9, 65).

Guidelines for control

The absence of reports of M. tuberculosis in free-ranging primates, elephants and psittacine birds suggests that M. tuberculosis is primarily a disease of human origin and that these animal groups are accidental hosts. Nonetheless, M. tuberculosis can be transmitted between these animals and humans and must be considered zoonotic. In humans, tuberculosis is most likely to be contracted in situations where prolonged contact occurs with infected individuals. This is also likely to be the case with these potential source species, suggesting that humans with daily, close contact with infected animals are at greatest risk. Psittacine pets may be a sentinel for human tuberculosis and could create a perpetuating reservoir of untreated M. tuberculosis infection (76). Any individuals in contact with elephants and primates should be tuberculin tested annually following established protocols for testing humans. All new employees should be medically examined and tested (with a tuberculin test or radiographically, depending on the conditions) prior to contact with primates and elephants. Anyone with active tuberculosis should not work with any animals until the disease is in remission. Recommendations for employee safety and health are included in the Guidelines for the Control of Tuberculosis in Elephants (71).

Other health programmes for zoo personnel address the prevention of transmission of tuberculosis from primates and elephants, in addition to other zoonotic diseases. Such programmes have recently arisen from zoo organisations and regulatory bodies in North America and Europe (44). Some examples are the quarantine procedures of the American Zoo and Aquarium Association (43) and the Infectious Disease Reviews of the American Association of Zoo Veterinarians (AAZV) (2). These contain information applicable to prevention of tuberculosis and other zoonotic diseases. Another strong deterrent, particularly in the group most exposed to zoonotic threats (e.g. keepers and veterinary staff) is a preventive health programme for zoo personnel established through the AAZV. The specific guidelines include preparing for emergency situations pertinent to the zoo environment and the identification of appropriate human health facilities and physicians familiar with the zoonotic diseases that might occur. Comprehensive preventive measures include tuberculosis and serological screening, serum banking and appropriate prophylaxis (60).

Some zoos have developed in-house protocols for specific zoonotic issues. For example, at the Smithsonian National Zoological Park in Washington, DC, an 'Elephant/People Interaction Protocol' has been established to limit direct contact between elephants and humans other than keepers and curatorial staff. The principal reason is to prevent the transmission of human tuberculosis from visitors and other individuals making 'behind the scenes' tours to the elephants. The popular communication methods such as blowing into the trunk and rubbing the tongue of the elephant are not permitted by outside visitors and are discouraged in the elephant caretakers.

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Directives for the surveillance and safeguard measures against some zoonotic diseases at zoos in Switzerland have been initiated via the Swiss Federal Veterinary Office (11). These address only the regulated diseases, such as tuberculosis, which principally apply to food and fur species. However, voluntary programmes to limit zoonoses have been established by individual zoos using quarantine methods, routine clinical and pathological surveillance, vaccination and parasite control. Finally, the European Association of Zoo and Wildlife Veterinarians has drafted guidelines on non-human primate zoonoses (12) which have been adopted by the Office International des Epizooties in the *International Animal Health Code* (48). This essentially involves both pre-export health certification and post-arrival quarantine with guidelines for tuberculosis and other zoonoses prevalent in non-human primates.

Conclusions

In contrast to *M. bovis*, *M. tuberculosis* has a more limited host range and does not appear to have an indigenous animal host or reservoir. This conclusion is principally based on the facts that *M. tuberculosis* does not appear to exist in the wild as an animal pathogen and that animals that do become infected appear to be accidental hosts. Nevertheless, if introduced into a primate research colony or exhibit facility, *M. tuberculosis* can be harmful, entailing high costs and the potential loss of rare and endangered species, as well as constituting a biohazard to attending personnel.

The emergence of *M. tuberculosis* in Asian elephants in North America in 1996 illustrates the way in which an insidious disease which has been present in the Asian elephant population for centuries can have a relatively sudden impact. This impact is heightened by the fact that the species is already threatened in the wild and is struggling to maintain numbers in captivity. However, action by veterinary and public health authorities, in co-operation with the zoological veterinary community in the USA, rapidly formulated a public programme to control this agent of human tuberculosis in elephants. This was accomplished in a manner that has allowed for the treatment and rehabilitation (rather than depopulation) of these endangered animals while diminishing the zoonotic hazard for humans.

Further possibilities of animal infections, particularly with the global increase of *M. tuberculosis* in human populations, should be met with awareness, vigilance and a continued interest and co-operation in developing preventive programmes against this disease. Development of uniform and valid tests for the ante-mortem diagnosis of tuberculosis in many of these exotic species should be vigorously pursued. Complete post-mortem examinations must always be performed with cultures and histopathological examination, particularly in targeted species, in an attempt to rule out mycobacterial diseases.

These actions, some of which are underway in the USA, will benefit both these important non-domestic, tuberculosis-susceptible species and humans.

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Mycobacterium tuberculosis chez les animaux sauvages et dans les parcs zoologiques

R.J. Montali, S.K. Mikota & L.I. Cheng

Résumé
La tuberculose due à *Mycobacterium tuberculosis* et à des agents apparentés à *M. tuberculosis* a été identifiée chez de nombreuses espèces, dont des primates non humains, des éléphants et autres ongulés, des carnivores exotiques, des mammifères marins et des psittacidés. L'infection due à *M. tuberculosis* concerne essentiellement les animaux en captivité et ne semble pas se déclarer naturellement chez les mammifères en liberté. Si c'est probablement chez l'homme que l'infection à *M. tuberculosis* a trouvé son origine, en tant qu'agent de zoonose le micro-organisme peut être transmis en retour à l'homme par les primates non humains, les éléphants d'Asie et les psittacidés. Cependant, la prévalence globale de la maladie chez ces espèces susceptibles est faible et les cas démontrés de transmission de *M. tuberculosis* des animaux à l'homme restent rares. *Mycobacterium tuberculosis* provoque des maladies pulmonaires

évolutives chez les mammifères et une infection muco-cutanée chez les perroquets. Dans tous les cas, le micro-organisme peut être éliminé dans le milieu extérieur et s'y propager. Chez les animaux vivants, le diagnostic repose sur l'épreuve de tuberculination intradermique pour les primates non humains, sur la mise en culture des sécrétions nasales pour les éléphants et sur la biopsie et la mise en culture de prélèvements des lésions externes dans le cas des perroquets. Des tests complémentaires, au moyen de sondes d'acide désoxyribonucléique ou par amplification de l'acide nucléique, ou encore des épreuves immuno-enzymatiques ont été adaptés à certaines de ces espèces avec des résultats encourageants. De plus, de nouvelles directives ont été élaborées aux États-Unis d'Amérique pour lutter contre la tuberculose chez les éléphants et des programmes ont été mis en place pour prévenir la maladie chez le personnel chargé des soins des animaux.

Mots-clés
Animaux de parcs zoologiques – Faune sauvage – Maladies animales – Mycobactéries – Mycobacterium tuberculosis – Tuberculose – Zoonoses.

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Mycobacterium tuberculosis en la fauna salvaje y de parques zoológicos

R.J. Montali, S.K. Mikota & L.I. Cheng

Resumen
La tuberculosis causada por *Mycobacterium tuberculosis* u otros microorganismos afines se ha detectado en especies animales muy diversas, desde primates no humanos, elefantes y otros ungulados y carnívoros exóticos hasta mamíferos marinos o psitácidos. La enfermedad asociada a ese patógeno se ha manifestado sobre todo entre animales en cautividad, y no parece presentarse de forma natural entre los mamíferos en libertad. Aunque es probable que *Mycobacterium tuberculosis* se originara como infección del ser humano, en términos zoonóticos tienen capacidad de transmitir esa enfermedad al hombre los primates no humanos, los psitácidos y el elefante asiático. No obstante, la prevalencia total de la enfermedad entre dichas especies susceptibles es baja, y los casos descritos de transmisión de *M. tuberculosis* de animales al hombre son infrecuentes. *Mycobacterium tuberculosis* provoca una enfermedad pulmonar progresiva en los mamíferos y una dolencia mucocutánea en el loro. En todos los casos, la enfermedad puede difundirse y esparcirse en el entorno. En animales vivos, el diagnóstico se fundamenta en la prueba tuberculínica intradérmica (en el caso de primates no humanos), el cultivo de secreciones nasales (en el elefante) o la biopsia y cultivo de lesiones externas (en el loro). También se ha intentado, con resultados prometedores, adaptar a algunas de esas especies técnicas basadas en sondas de ácido desoxirribonucleico y amplificación de ácido nucleico, así como técnicas inmunoenzimáticas, utilizándolas a modo de pruebas complementarias. Se han elaborado además nuevas directrices para controlar la tuberculosis en elefantes en los Estados Unidos de América, y se han puesto en marcha programas para prevenir la enfermedad entre las personas que trabajan en contacto con animales.

Palabras clave
Animales de zoológico – Enfermedades animales – Fauna salvaje – Micobacterias – Mycobacterium tuberculosis – Tuberculosis – Zoonosis.

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