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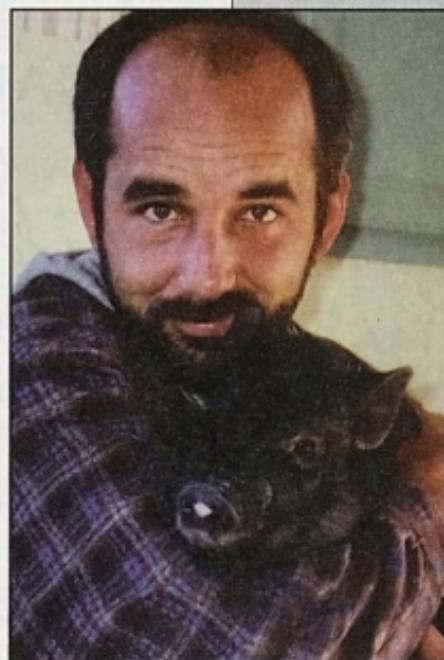
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### About the Cover

*The Eye Exam was painted in 1995 by Dr. Robin Peterson. Dr. Peterson graduated from Washington State University in 1981, after which she did a surgical residency at the University of California-Davis. Recently, she left active practice in southern California to return to the Pacific Northwest (Gig Harbor, Wash), where she is pursuing a career in freelance illustration and portrait work.*

*The children depicted in the painting are those of the authors of the review article titled "Vision in dogs," which begins on page 1623. Permission to adapt the UC-Berkeley School of Optometry poster for inclusion in the painting was granted by the poster's creator, David Goines.*

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## Epizootic of *Mycobacterium bovis* in a zoologic park

Mark D. Stetter, DVM; Susan K. Mikota, DVM; Andrew F. Gutter, DVM; Edgar R. Monterroso, MD, MPH; Joseph R. Dalovisio, MD; Charles Degraw; Thomas Farley, MD, MPH

- Tuberculosis is a reemerging zoonotic disease with a wide range of potential hosts. *Mycobacterium bovis* can cause illness and death in infected animals.
- Zoo personnel in contact with infected animals can develop positive results on intradermal tuberculin skin tests.
- The lack of reliable diagnostic tests and effective treatments for nondomestic species continues to make tuberculosis a serious threat to human beings and other animals.

A 32-year-old female southern white rhinoceros (*Ceratotherium simum simum*; rhinoceros 1) was examined to determine the cause of intermittent ocular and nasal discharge during an 11-year period. The clinical signs had a seasonal pattern and were suspected to be allergy related. Various antibiotics had been administered po during the 11 years, but the efficiency had been questionable. In January 1989, rhinoceros 1 was tuberculin tested, using purified protein derivative (PPD)-bovis antigen administered intradermally in the caudal tail fold. The test result was negative at 24 hours, but a diffuse dermal swelling was observed at 48 and 72 hours. In September 1989, rhinoceros 1 was chemically immobilized to enable evaluation of the continued chronic respiratory tract infection. Immobilization was accomplished by use of 2.5 mg of etorphine administered IM via projectile dart. After completion of the examination, etorphine was successfully reversed by use of 4 mg of diprenorphine administered IV and 4 mg of diprenorphine administered SC. Rhinoscopy and bronchoscopy were performed, and multiple biopsy specimens were obtained for cytologic and histologic examination and for bacterial culturing. Hematologic examination and intradermal tuberculin skin testing also were performed. Comparative tuberculin tests were done, using PPD-bovis and PPD-avian antigens administered in 3 locations (caudal tail fold, base of ear pinna, and antebrachium). All sites were interpreted as non-reactive at 48 and 72 hours, except for the PPD-bovis injection site in the caudal tail fold. Examination of Ziehl-Neelsen stained slides of nasal and tracheal epithelial samples did not reveal acid-fast bacilli bacteria, and aerobic bacterial and mycobacterial culture results

were negative. In December 1990, rhinoceros 1 was immobilized for examination of the reproductive tract, but the rhinoceros died of anesthetic-related causes. Immobilization was accomplished by use of 3 mg of etorphine administered IM via projectile dart, followed by 100 mg of xylazine hydrochloride administered IV. Gross necropsy findings and results of histologic and cytologic examination and of mycobacterial culturing did not yield evidence of mycobacterial disease.

A 29-year-old male rhinoceros (rhinoceros 2) had an unremarkable medical history. In January 1989, rhinoceros 2 was tuberculin tested, using PPD-bovis antigen administered at the base of the ear pinna. The site was interpreted as negative at 24, 48, and 72 hours. In April 1989, rhinoceros 2 was tuberculin tested again, using PPD-avian and PPD-bovis antigens. The PPD-avian antigen site was interpreted as negative, but a small firm swelling at the PPD-bovis antigen site was detected via palpation. In April 1991, rhinoceros 2 became ill. Clinical signs included loss of weight, stranguria, an intermittent productive cough, and bilateral mucopurulent nasal discharge. Results of a CBC, serum biochemical analysis, urinalysis, and aerobic bacterial culturing of nasal discharge were all nondiagnostic. Rhinoceros 2 progressively deteriorated, and the rhinoceros died in June 1991. Gross necropsy revealed a severe granulomatous pneumonia. Histologic examination of Ziehl-Neelsen stained sections revealed numerous acid-fast bacilli bacteria in the pulmonary granulomas and thoracic lymph nodes. *Mycobacterium bovis* was isolated from the granulomas.<sup>a</sup>

A 16-year-old male southern white rhinoceros (rhinoceros 3) and a 16-year-old female southern white rhinoceros (rhinoceros 4) were clinically normal. In January 1989, rhinoceros 3 and 4 were tuberculin tested, using mammalian old tuberculin and PPD-bovis antigens administered at the base of each ear pinna. Both sites in rhinoceros 3 were interpreted as negative at 24, 48, and 72 hours. Rhinoceros 4 would not allow evaluation at 24 or 48 hours, but the PPD-bovis antigen site was negative at 72 hours, whereas the mammalian old tuberculin antigen site had a diffuse swelling. In April 1989, rhinoceroses 3 and 4 were tuberculin tested again, using injections of PPD-avian and PPD-bovis antigens in the caudal tail fold. Both sites were negative at 24, 48, and 72 hours in rhinoceros 3, but diffuse swelling (3 to 5 cm) was seen at both antigen sites in rhinoceros 4.

Because of their exposure to *M bovis*, rhinoceroses 3 and 4 were chemically immobilized for evaluation in September 1991. Rhinoscopy, bronchoscopy, hematologic tests, percutaneous lung biopsy, and intradermal tuberculin skin testing were performed. Injections of PPD-avian, PPD-bovis, and mammalian old tuberculin antigens were administered at several test sites in each

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rhinoceros. All sites in both rhinoceroses were interpreted as negative at 24, 48, and 72 hours. Three days after immobilization, rhinoceros 3 died from exertional rhabdomyolysis and renal failure. Rhinoceros 4 was considered to be at high risk of developing tuberculosis and was considered to be a potential health threat to other zoo animals, zoo personnel, and the general public. Rhinoceros 4 was euthanatized. Gross necropsy, histologic examination that included use of Ziehl-Neelsen stains, and mycobacterial culturing did not reveal any evidence of mycobacterial disease in rhinoceros 3 or 4.

In August 1991, a 2-year-old male colobus monkey (*Colobus guereza caudatus*; monkey 1) was examined, because it was mildly lethargic, weak, and partially anorectic. Monkey 1 had been tuberculin tested 10 months previously, and results were considered negative at that time. A CBC and serum biochemical analysis as well as intradermal tuberculin skin testing, thoracic and abdominal radiography, and ultrasonography were performed. Radiography revealed a severe pericardial effusion, and an ultrasound-guided pericardial centesis was performed. Cytologic examination of Ziehl-Neelsen stained fluid samples revealed numerous acid-fast bacilli bacteria. Subsequently, *M bovis* was isolated. Intradermal tuberculin skin testing was performed, using mammalian old tuberculin and avian old tuberculin antigens administered in the right and left eyelids, respectively. The mammalian old tuberculin antigen site was interpreted as positive on visual examination at 48 and 72 hours. The avian old tuberculin antigen site was negative at 24, 48, and 72 hours on visual examination. On the basis of cytologic exam findings and intradermal tuberculin skin testing, a diagnosis of pulmonary tuberculosis was made.

In September 1991, a 6-year-old male colobus monkey (monkey 2) was examined because of lethargy and weakness. Initial diagnostic evaluation was similar to that of monkey 1. Ultrasonography revealed diffuse granulomatous disease with pulmonary, hepatic, and renal involvement. Results of examination of Ziehl-Neelsen stained cytologic samples obtained by means of abdominocentesis were positive for acid-fast bacilli bacteria, and *M bovis* was isolated subsequently. A slight swelling was evident at the mammalian old tuberculin antigen test site at 48 and 72 hours and was interpreted as a suspicious reaction. The test site injected with avian old tuberculin antigen was interpreted as negative. A diagnosis of disseminated tuberculosis was made.

After diagnosis, monkeys 1 and 2 were placed in isolation facilities and were started on treatment with rifampin (150 mg, PO, q 24 h) and isoniazid (100 mg, PO, q 24 h). Despite treatment, monkey 1 continued to deteriorate and died in October 1991. One month after the onset of illness, monkey 2 was tested again for tuberculosis. At that time, mammalian old tuberculin antigen was administered in the right eyelid, and PPD-bovis antigen was administered in the left eyelid. Both intradermal tuberculin skin test sites were visually interpreted as nonreactive at 24, 48, and 72 hours. Despite treatment, monkey 2 continued to do poorly, and it was decided that it should be euthanatized in December 1991. Forty-eight hours prior to euthanasia, monkey 2 was tuberculin tested again with injections intradermally of mammalian old tuberculin and PPD-bovis antigens. At

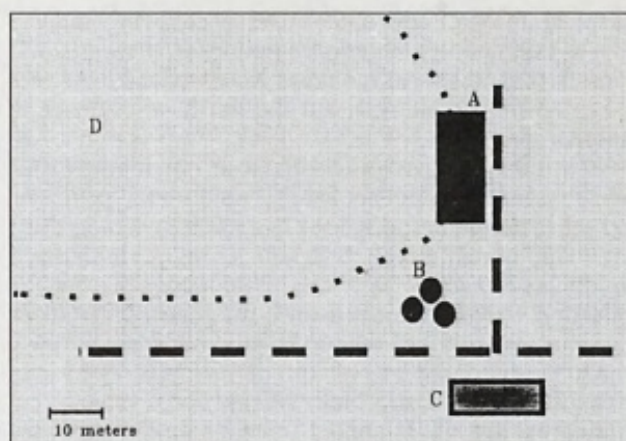


Figure 1—Illustration depicting the housing facilities of the animals involved in an epizootic of *Mycobacterium bovis* in a zoologic park. A = rhinoceros barn; B = colobus monkey outdoor holding area (monkey 1); C = colobus monkey exhibit area (monkey 2); D = rhinoceros exhibit area; ■■■ = chain link fence and wooden posts (1.5 m high) surrounding rhinoceros exhibit area; — = service road.

necropsy, the test sites were visually interpreted as suspicious for the mammalian old tuberculin antigen and nonreactive for the PPD-bovis antigen. Histologic examination of the mammalian old tuberculin antigen test site revealed inflammatory lesions compatible with a positive reaction to the tuberculin antigen. Inflammatory lesions at the PPD-bovis antigen site were mild and were considered to be the result of a reaction to the vehicle rather than the antigen.

Deoxyribonucleic acid fingerprinting was done to compare *M bovis* strains from rhinoceros 2, monkey 1, and monkey 2.<sup>b</sup> Results indicated that all 3 isolates were genetically similar and that a single mycobacterial strain was probably responsible for the infections in all 3 animals.

The rhinoceros facility was a one-story building located adjacent to a large outdoor field exhibit area. Indoor quarters were separated into 6 stalls by large metal bars. The floor, walls, and roof were made of cement, and the building had 3 doors and a large ceiling vent with an exhaust fan. Monkey 1 had been housed with 2 other colobus monkeys in a series of outdoor cages, which were located approximately 15 m from the rhinoceros building and 8 m from the rhinoceros exhibit area (Fig 1). Dense foliage separated the monkey and rhinoceros facilities. Monkey 2 had been housed with 4 other colobus monkeys in an indoor facility that had an outdoor public exhibit area. This indoor facility was located approximately 30 m from the rhinoceros area. Monkey 2 did not have direct contact with the other animals with tuberculosis (monkey 1 or rhinoceros 2). After tuberculosis was diagnosed, all exposed nonhuman primates were quarantined in their respective housing facilities. This included 6 colobus monkeys (2 housed with monkey 1 and 4 housed with monkey 2) and 7 howler monkeys (*Alouatta* sp) that were housed in the same area (2 housed with monkey 1 and 5 housed with monkey 2). Evaluations performed on the monkeys included physical examination, intradermal tuberculin skin testing, thoracic radiography, CBC, and serum bio-



chemical analysis, and mycobacterial culturing and cytologic examination of samples obtained by means of a transtracheal wash. All monkeys were evaluated 3 times at 1- to 3-month intervals but did not have evidence of tuberculosis.

The zoologic garden described in our report maintained various exotic hoofstock, nonhuman primates, and other nondomestic species in a modern zoologic setting. The animal health program strongly emphasized prophylactic medical care, and all animals entering the collection were quarantined for a minimum of 30 days. All primates were given routine physical examinations, which included the use of intradermal tuberculin skin testing. Old World and New World monkeys were examined on arrival, at annual intervals, and when they had signs of illness. Lowland gorillas (*Gorilla gorilla gorilla*) and orangutans (*Pongo pygmaeus*) were chemically immobilized for physical examination on arrival and at 2-year intervals. Mammalian old tuberculin and avian old tuberculin antigens were used in the right and left eyelids, respectively, for intradermal tuberculin skin testing of primates.

Hoofstock were examined during the quarantine period and when opportunities arose. Hoofstock species were tuberculin tested with 1 or more tuberculin antigens. Mammalian old tuberculin and PPD-bovis antigens were used most frequently, but avian old tuberculin and PPD-avian antigens also were used.

Unlike other hoofstock species at the zoologic garden, the rhinoceroses historically were considered to be tuberculin reactors. Interpretation of intradermal tuberculin skin tests in rhinoceroses is difficult, and results are often unreliable.<sup>1-4</sup> The zoologic garden maintained 4 adult (2 male and 2 female) southern white rhinoceroses that originally had been imported from Africa. New rhinoceroses had not been introduced into the herd during the 16 years prior to the epizootic. Rhinoceroses were housed in a common building and shared an outdoor exhibit area. None of the rhinoceroses had previously had major medical problems, although 1 of the rhinoceroses did have a history of nasal discharge on a seasonal basis, which was believed to have been allergy related.

With the exception of the orangutans, none of the primates historically were considered to be tuberculin reactors. Orangutans are nonspecific reactors to the tuberculin antigen, and a positive result on an intradermal tuberculin skin test does not necessarily correlate with disease.<sup>3-5</sup> The orangutans in the zoologic collection were all in good health and did not have radiographic or clinical evidence of mycobacterial disease.

All animal department personnel were required to have a preemployment intradermal tuberculin skin test and annual tests thereafter. The animal department employed 2 people that did have a history of having positive results on intradermal tuberculin skin tests. These people were given biannual physical examinations that included thoracic radiography, but evidence of active disease was not detected in these people. Volunteers who worked closely with primates also were required to have a yearly test for tuberculosis. Other volunteers and personnel in departments other than the animal department were not routinely tested.

Twenty-four zoo personnel that had been exposed

to the tuberculosis-infected rhinoceros were identified. These people had been tuberculin tested in January 1991, using the tine test PPD,<sup>6</sup> and results were negative at that time. In June 1991 after tuberculosis was diagnosed in rhinoceros 2, further testing was done. Twenty-three people (hoofstock keepers, curators, and veterinary staff) were given intradermal tuberculin skin tests, using 5 units of intermediate-strength PPD (Mantoux test).<sup>6</sup> Testing was done by the infectious disease unit of a local hospital. Two people had a positive result on the skin test. In July 1991, intradermal tuberculin skin testing was repeated, and 1 additional person was detected as a reactor. Four weeks later, all people that had been exposed were tested again, and 4 additional people had results that were considered positive. Six of the 7 people with positive skin-test results were zookeepers who had been responsible for the daily feeding and cleaning of the rhinoceroses. The seventh person was an animal curator who had been exposed infrequently to rhinoceros 2 but who had assisted with the necropsy.

All employees who had positive results on intradermal tuberculin skin tests were evaluated further by a physician. A CBC, serum biochemical analysis, determination of an erythrocyte sedimentation rate, urinalysis, and thoracic radiography were performed. None of the tuberculin reactors had evidence of active disease, and they were placed on antibiotics (300 mg of isoniazid, PO, q 24 h, for 9 months) prophylactically. During that time, 3 of 7 people did not finish the medication regimen. One year after the positive result on the intradermal tuberculin skin test, none of the people had developed any clinical signs of tuberculous disease.

Daily cleaning of stalls in the rhinoceros barn included manual removal of feces with a shovel, after which high-pressure washing of the floors and walls was performed. Inadequate ventilation combined with high-pressure washing may have caused infectious biomaterial to aerosolize during cleaning. Prior to the death of the tuberculosis-infected rhinoceros, workers in the facility did not wear masks, gloves, or protective face shields. After the diagnosis of tuberculosis, zookeepers were instructed to wear respirators fitted with high-efficiency particulate aerosol filters. Daily disinfection with a phenol detergent<sup>d</sup> was added to the routine cleaning procedures for the rhinoceros building. Similar preventative health measures were instituted for workers in the colobus monkey facilities.

The epizootic attributable to infection with *M bovis* at this zoologic park was believed to have originated from a single infected rhinoceros. Rhinoceros 2 may have become infected in Africa but did not become clinically ill until 15 years later. A similar late reactivation of tuberculosis has been reported for human beings.<sup>7</sup> Infants are exposed and infected when young but may not become ill until they are much older. A decline in the immune system is believed to trigger an intracellular dormant mycobacterium organism to begin a reproductive cycle. Adult onset reactivated tuberculosis is predominately a pulmonary disease, similar to that seen in rhinoceros 2, and a similar pathogenesis has been described in other animals.<sup>7</sup> Authors who reported that rhinoceroses developed clinical tuberculosis after long periods in captivity speculated that the rhinoceroses were originally infected in the wild.<sup>1</sup>



It is uncertain how the bacteria may have spread from the rhinoceros building to the primate areas. Fomites rarely play an important role in the transmission of tuberculosis in human beings, and instruments and tools were not used in more than 1 area. Similarly, personnel that cared for the rhinoceroses did not enter primate areas. People who were permitted to have close contact with the rhinoceroses and the monkeys had been uniformly tested for tuberculosis with negative results. Other personnel who had positive test results did not have evidence of shedding of the organism or signs of clinical disease. The most likely route of spread appeared to be via aerosolization between separate housing areas. Bacilli-filled droplet nuclei may have been formed when the rhinoceros area was being washed. Droplet nuclei may have been carried out through the ceiling fan and traveled to the adjacent outdoor primate facilities. This type of aerosol transmission is the primary mode of spread in cattle.<sup>4,8</sup> Although tubercle bacillus can be deactivated readily by ultraviolet light, when kept moist in droplet nuclei, they are shielded from the effects of ultraviolet light.<sup>8,9</sup> The design of the rhinoceros building, high local humidity, potentially high number of organisms being shed, and use of pressure washing all may have contributed to the unusually distant spread reported here. New World monkeys, great apes, and human beings have enzymes that provide a more effective macrophage defense against the mycobacterial bacillus organism and, thus, they are more resistant to mycobacterial infection, compared with Old World monkeys.<sup>9,10</sup> This may explain why the colobus monkeys (Old World) became ill, whereas the howler monkeys (New World) housed in the same area did not.

The high number of positive test results in zookeepers exposed to the infected rhinoceros indicated a high degree of exposure. Rhinoceros 2 was believed to have been shedding a large number of organisms in its nasal discharge, saliva, and, possibly, in its feces and urine. If rhinoceros 2 was shedding organisms from the time it was first documented as being ill, human beings and other animals may have been exposed for more than 60 days.

In modern zoologic parks, tuberculosis is usually more of a diagnostic dilemma than a clinical disease problem.<sup>11</sup> The description provided here documented some of the problems associated with the diagnosis of tuberculosis in nondomestic animals. Three of the rhinoceroses did not have detectable mycobacterial infection, yet all had previously had a response to intradermal tuberculin skin testing. Rhinoceros 2, with a confirmed *M bovis* infection, had negative test results 2 years prior to its death but was believed to have been harboring the organism at that time. Even the nonhuman primates for which the intradermal tuberculin skin test is considered to be reliable<sup>11</sup> had variable test results. Monkey 2 had only a mild swelling when tested at the onset of illness and did not have a reaction when tested a month later. Histologic examination during necropsy did not reveal an immune response to the PPD-bovis antigen at 48 hours after injection; however, *M bovis* was cultured

from granulomas in the thorax. Response to the mammalian old tuberculin antigen at 48 hours after injection was not a characteristic type-IV hypersensitivity reaction, as determined on the basis of histologic evaluation. The lack of a positive intradermal tuberculin skin test or characteristic histologic changes may have been a result of disease or chemotherapy-induced immunosuppression.<sup>12</sup> The severely debilitating condition associated with tuberculosis in monkeys can cause anergy and a negative result on an intradermal tuberculin skin test.<sup>11</sup> Isoniazid therapy also can cause a negative result on intradermal tuberculin skin testing in monkeys.<sup>12</sup>

Although authors have previously implicated *M tuberculosis* as the most important of the mycobacteria that involves human beings at zoos,<sup>8</sup> our report and others<sup>1,11,13</sup> were suggestive that *M bovis* is the most common mammalian tuberculosis problem in zoologic parks. The zoonotic potential, lack of reliable diagnostic tests, and wide range of potential hosts continue to make tuberculosis a threat to human beings and a tremendous number of other animal species.

<sup>a</sup>USDA, National Veterinary Services Laboratory, Ames, Iowa.

<sup>b</sup>Centers for Disease Control, Atlanta, Ga.

<sup>c</sup>Lederle Laboratories, Wayne, NJ.

<sup>d</sup>One-stroke environ, Calgon Vestal Laboratories, St Louis, Mo.

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