CHAPTER 2

Current Diagnostic Methods for Tuberculosis in Zoo Animals

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Tuberculosis (TB) is a cause of significant morbidity and mortality in both domestic and wild animals worldwide. Although a wide variety of mycobacteria are pathogenic in mammals, birds, reptiles, amphibians, and fish, "tuberculosis" refers to infection with specific organisms belonging to the *Mycobacterium tuberculosis* complex. The presence of TB in zoologic collections has been documented for at least 100 years and suspected to affect wildlife species even longer.

The interaction of free-ranging wildlife and domestic livestock in many countries has led to complex disease issues regarding the control of TB. Furthermore, the zoonotic potential of these organisms presents an additional concern for animal handlers and the public. Therefore, rapid, accurate diagnosis in wildlife species is important not only to zoo veterinarians, but also to those responsible for managing wildlife, to regulatory bodies, and to the public.

ETIOLOGY

The TB complex includes *Mycobacterium tuberculosis, M. bovis, M. africanum, M. microti,* and *M. pinnipedii.*[11,14] *M. tuberculosis* is the predominant cause of TB in humans and elephants, whereas *M. bovis* is the most common cause of TB in domestic animals and wild mammals.[30] *M. microti* is primarily found in small rodents (voles) and hyraxes but has also been isolated from llamas, pig, and ferrets. *M. africanum* is a rare cause of TB in humans, cattle, and pigs.

Mycobacterial classification has typically relied on biochemical and phenotypic characteristics of the organisms. These bacteria are slow growing and take up to 8 weeks to appear on Löwenstein-Jensen media cultured aerobically at 37° C. Culture morphology varies from coccoid to filamentous, and microscopically the rod-shaped bacteria are 0.2 to 0.6 μm × 1.5 to –3.0 μm. Presumptive identification of mycobacteria may be made by demonstrating acid-fast staining characteristics using Ziehl-Neelsen or the Kinyoun staining techniques with carbolfuchsin. In addition to biochemical differentiation, deoxyribonucleic acid (DNA)–specific probes have been developed to provide speciation.[1,39] Strains have also been identified within species using restriction fragment length polymorphism (RFLP) of identified sequences, spoligotyping, and DNA sequencing.[30,33]

DIAGNOSTIC TESTS

No antemortem test is 100% reliable for detecting TB in zoo animals. The approach to routine screening and clinical examination of suspect cases requires application of multiple testing modalities. It is important to realize that most tests are not validated in zoo animal species, and those based on immunologic responses especially may show significant variability among species. As technology and knowledge expand, the ability to interpret these tests will increase, but until then the clinician using these diagnostic methods is advised to use caution and understand the potential limitations of each test. A brief synopsis of current diagnostic test modalities follows; the reader is advised to refer to more extensive literature reviews on the subject.

Testing Based on Detection of Mycobacterial Organisms

Diagnostic tests that identify the mycobacterial organism, or components, are the most definitive method of detecting infection. Culture and speciation is considered the "gold standard" and also takes the longest to obtain results (up to 8 weeks, or more for

speciation). Even in human cases, infection is only demonstrated in 50% of adult cases by proof of bacilli in biologic samples.[38] Site of infection, intermittent shedding, and difficulty of obtaining samples from some species may lead to decreased recovery of organisms. Laboratories with expertise in mycobacterial culture should be chosen when submitting samples. If treatment is being considered in highly valuable or endangered individuals, culture is necessary for identification and antibiotic sensitivity testing. Improved culture methods, such as at BACTEC, Septi-Chek, MB/BacT systems, and mycobacterial growth indicator tubes (MGITs), have the potential to decrease time to detection of growth and increase rate of recovery.[31]

Direct staining of sample material may provide presumptive identification as acid-fast bacteria, but there are also nonmycobacterial organisms, such as *Nocardia,* that may stain positive. Immunohistochemical staining of tissues is also useful for antemortem diagnosis in limited cases in which biopsy or other relevant samples (e.g., lymph node) may be available. Labeled monoclonal antibodies may confirm acid-fast organisms in tissues as being mycobacteria.

Amplified *M. tuberculosis* direct test (MTD) and multiplex polymerase chain reaction (PCR) assays may provide rapid results by detecting nucleic acid from the organism in clinical samples.[33,39] Gene probes are used for rapid identification of mycobacterial isolates, whereas the gene amplification methods such as PCR are used to aid in identification of species as well as to test culture-negative samples.[30,39] By choosing the appropriate primers, PCR tests may distinguish between *M. tuberculosis* complex and *M. avium.*

PCR may also be performed on postmortem samples, including formalin-fixed tissues.[39] A combination of techniques was compared for postmortem detection of *M. bovis* in white-tailed deer *(Odocoileus virginianus).* Histopathology had a positive predictive value (PPV) of 94%, acid-fast staining had a PPV of 99%, and application of an *M. tuberculosis* group-specific genetic probe had 100% PPV compared with mycobacterial culture.[20]

Secreted antigens from proliferating mycobacteria have been the focus of recent diagnostic research. *Antigen 85* (Ag85), produced during active infection, has been detected in sera using dot blot immunoassay. Nyala *(Tragelaphus angasi)* with pulmonary granulomatous lesions had elevated values of Ag85 compared to those with no history of exposure to *M. bovis.*[36] However, similar tests on orangutans showed equivocal results.[32] Serum Ag85 could be used as an adjunct test but appears to require further validation in each species.

Testing Based on Immunologic Response to Mycobacteria

Cell-Mediated Immunologic Tests

The most common diagnostic test for TB in mammals is the intradermal test, based on in vivo, delayed-type hypersensitivity response to tuberculin antigens. Purified protein derivative (PPD) tuberculins prepared from *M. bovis* and *M. avium* are used for single and comparative testing, particularly of ungulate species.[30] The standard dose is 0.1 mL (5000 tuberculin units) in mammals, injected intradermally, usually in the caudal tail fold, skin of the cervical region, or upper eyelid of primates. Other sites used include the lateral thorax, axillary region, abdomen, and ear. Old tuberculin (OT), prepared from either *M. tuberculosis* or *M. bovis,* has historically been used in primates and zoo ungulates but has been phased out because it is more difficult to standardize between lots and is less specific. Currently, most PPD tuberculin is produced at a protein concentration of 1 mg/mL.[30] Ideally, injection sites are measured with calipers at initial injection and again after 48 hours in nonhuman primates and swine or after 72 hours in ungulates. Specific criteria for "negative" and "suspect" have been developed only for a few nondomestic species, including some cervids. If swelling is present, additional diagnostic testing, including a *comparative cervical test* (CCT), is warranted. Ancillary tests, such as the interferon-gamma (IFN-γ) test, have been approved in the U.S. federal eradication program for domestic cattle to replace or augment the results of CCT. The basis of the CCT is that there will be a differential response to *M. avium* and *M. bovis* PPD based on whether the animal is infected with *M. tuberculosis* complex or has had a transitory sensitization from nontuberculous mycobacteria.

Intradermal testing is fraught with problems, including anergic responses in individuals with fulminant disease, species and individual variability in response, and false-positive and false-negative reactions. Even in humans, the positive predictive value for tuberculin skin test varies with infection prevalence in the tested population, with at least a PPV greater than 75% in which infection prevalence was above 10%, but decreased PPV in populations with lower prevalence.[3] Certain zoo species are known to have an increased likelihood of nonspecific reactions, including tapirs, bongo antelope, reindeer, and orangutans. To address these issues, the use of purified antigens in vivo and in vitro is being investigated in a variety of species.

Diagnostic tests based on in vitro cell-mediated immune responses to mycobacteria include lymphocyte transformation, cytokine production (i.e., IFN-γ, interleukin-2), and other indirect measures of immunologic stimulation, such as cytokine ribonucleic acid (RNA) assays. *Lymphocyte transformation* (LT) tests are performed by stimulating mononuclear cells with specific antigens and then incubating the proliferating cells with a radioisotope-labeled nucleotide. The amount of label incorporated is correlated with the degree of proliferation and is an indicator of previous exposure and immune recognition of the specific antigen. The LT assay was part of the blood tuberculosis (BTb) test developed to overcome the problems associated with skin testing and was used as an ancillary test for U.S. deer in the 1990s.[12] A similar comparative lymphocyte stimulation test developed for *M. bovis*–infected Eurasian badgers *(Meles meles)* using bovine and avian tuberculins showed 87.5% sensitivity and 84.6% specificity.[17]

Assays that measure cytokine production, such as IFN-γ and interleukin-2 (IL-2), appear to be more sensitive than skin tests. Cytokines are generally more conserved between species, so detection methods may be more widely applicable. For example, the immunoassay developed for human IFN-γ was able to detect chimpanzee, orangutan, gibbon, and squirrel monkey IFN-γ and correlated with in vivo tuberculin skin reactivity.[19] This test was commercially available as Primagam (CSL Veterinary, Australia) for use in gorilla, orangutan, chimpanzee, gibbon, guereza, mandrill, squirrel monkey, marmoset, and baboon. A similar assay was produced for cattle (Bovigam), deer (Cervigam), and humans (Quantiferon). The IFN-γ test has been used with African buffaloes *(Syncerus caffer)* to aid in a test and cull program for bovine TB in Kruger National Park, South Africa.[24] Necropsy and culture results were used to confirm field cases, and the specificity of the IFN-γ test was shown to be 99.3%. Recent research investigating other cytokine production (e.g., IL-2) or cytokine RNA may provide additional in vitro methods of assessing response to mycobacterial infection across a range of species.[44] Difficulties associated with using these assays include (1) specific culture parameters need to be developed for each species, and (2) whole blood needs to be properly handled for accurate test results. Many of these tests are not currently available on a commercial basis.

Serologic Tests

Enzyme-linked immunosorbent assay (ELISA) has been the most frequently used serologic test for TB diagnosis. These assays incorporate various forms of mycobacterial antigens for detection of antibodies in the test sample and also are a component of the BTb test. In one study of 12 cervid herds, the specificity and sensitivity of a five-antigen ELISA were 78.6% and 70.0%, respectively.[21] The ability to diagnose TB increased if ELISA and tuberculin skin test results were used in parallel, rather than using either test alone.

ELISA has been used to evaluate *M. bovis* infection in brushtail possums *(Trichosurus vulpecula)* in field tests.[6] The sensitivity and specificity of the assay using *M. bovis* culture filtrate was 45% and 96%, respectively, and the results were 21% and 98% when the antigen was MPB70. Further study showed that *M. bovis*–infected possums develop antibody late in the course of disease that may affect the sensitivity of serologic diagnostic tests for this species. This underscores the importance of understanding the immunologic response to TB in each species and the potential limitations of serologic assays.

With the development of purified, recombinant, and fusion proteins, tailored antigen panels may be developed to change specificity and sensitivity of serologic tests. In addition, other methods may be employed, such as Western blot (immunoblot), thin-layer immunochromatography, and multiantigen print immunoassay (MAPIA). Immunoblot has been demonstrated to be a sensitive method to detect and monitor development of serologic response to specific mycobacterial protein antigens in a variety of species.[49] Immunodominant antigens may be identified and used for development in other serologic assays, such as ELISA or immunoblot. MAPIA entails application of antigens to nitrocellulose membranes, followed by incubation with test sera and detection using standard chromogenic immunodevelopment.[35] MAPIA has been useful in choosing antigens appropriate for a rapid test that utilizes thin-layer immunochromatography and may provide a diagnostic screening test for field situations.[23]

In a study comparing serologic and cell-mediated responses to *M. bovis* in reindeer, antibody could be detected as early as 4 weeks after experimental infection.[49] Animals tested positive using multiple serologic tests but showed individual variation in antigen recognition at different time points. MAPIA appeared to be most sensitive and detected antibodies earliest after infection at 4 weeks, immunoblot at 8 weeks, and ELISA at 15 weeks. When compared with IFN-γ and skin test responses, all the infected reindeer tested positive by CCT at 3 and 8 months after infection, but no correlation was found between skin test reaction

and level of antibody. Similarly, there was no correlation between antibody levels and IFN-γ response. This study shows the potential diagnostic value of serologic tests in a species that has a low prevalence of disease and a high number of nonspecific reactions with skin testing.

CURRENT PROTOCOLS FOR ZOO ANIMALS

Tuberculosis, caused by *M. bovis* or *M. tuberculosis*, is a reportable disease in the United States. Worldwide, TB is one of the infectious diseases that causes the greatest annual morbidity and mortality in humans, with an estimated 2 to 3 million deaths each year.[30] TB has been diagnosed in most mammalian taxa typically housed in zoologic collections. Sporadic cases, as well as epizootics, have occurred in zoos around the world.[16,33,46]

The diagnosis of TB in a zoologic collection may lead to restriction of animal movement, issues associated with human health, and euthanasia of potentially healthy animals. To address these concerns, the National Tuberculosis Working Group for Zoo and Wildlife Species was established to develop protocols for testing and movement of zoologic species, with a focus on nondomestic hoofstock and elephants.[48] The protocol *Guidelines for the Control of Tuberculosis in Elephants* is available on the American Association of Zoo Veterinarians (AAZV) website (www.aazv.org); *Tuberculosis Surveillance Plan for Non-Domestic Hoofstock* is being finalized. Additional goals of the surveillance plan are to establish data on diagnostic methods and estimate the true prevalence and incidence of TB in zoologic collections.

Guidelines for testing primates are often based on standards developed by the World Organization for Animal Health (OIE), Centers for Disease Control and Prevention (CDC), and National Institutes of Health (NIH). Origin, history of close human contact, and environment are primary risk factors in determining likelihood of TB in nonhuman primates. Certain species and exposure to other mycobacteria have been correlated with an increase in false-positive skin reactions.[9]

More recently, the Veterinary Advisory Group of the Animal Health Committee of the Association of Zoos and Aquariums (AHC-AZA) have started to develop taxon-specific or species-specific recommendations for preshipment and preventive health protocols that include standardized diagnostics, such as TB testing. This approach may facilitate data collection for determining the validity of various diagnostic tests for TB.

CLINICAL FINDINGS

Tuberculosis should be on the differential list for any mammal that exhibits clinical signs of chronic weight loss or emaciation, weakness, dyspnea, cough, and enlarged lymph nodes. Unfortunately, many infected animals are asymptomatic until disease is advanced. Therefore, a proactive quarantine and routine screening program should be developed for each zoologic collection housing susceptible species.

Primates

Primates may be infected by *M. bovis, M. tuberculosis, M. avium,* and rarely, other nontuberculous mycobacteria. It is important that diagnostic tests differentiate pathogenic mycobacterial infections from potential cross-reactions caused by exposure to other nontuberculous mycobacteria. The most common method of screening nonhuman primates is intradermal testing. OIE recommends that all imported prosimians, callitrichids, New and Old World monkeys, gibbons, and great apes be tested at least two or three times at 2- to 4-week intervals during quarantine (OIE Terrestrial Animal Health Code, 2005). Nonhuman primates require 1000 to 10,000 times more tuberculin than humans to elicit a delayed hypersensitivity response.[9] Therefore, it is important to use products manufactured for nonhuman primates, with a minimum dose of 1500 tuberculin units/0.1 mL. The most common site for injection is the upper eyelid, which is examined visually at 24, 48, and 72 hours for degree of swelling and erythema. Other injection sites include arm, thorax, or abdomen, especially in smaller species such as callitrichids. Because mammalian OT is a nonuniform product that may vary between batches, nonspecific reactions may be observed in uninfected primates. Some newer recommendations have switched from using mammalian OT to mammalian PPD in the single intradermal test because content is more easily standardized in these preparations. Comparative tests using mammalian and avian PPD, along with ancillary tests, should be performed in any individual that has a suspect reaction.

Additional diagnostic tests include complete blood count (CBC); thoracic radiographs; mycobacterial culture (may be done from lesions and tracheal/gastric lavage); PCR/MTD; acid-fast staining of tracheal/gastric lavage, feces, or tissue; and immunoassays. Molecular techniques such as PCR/MTD and RFLP may be used to distinguish pathogenic mycobacterial infections from atypical infections that may cause a

positive tuberculin skin response. This method was used to identify asymptomatic *M. kansasii* infections in several squirrel monkeys that were suspect responders.[5] In a zoo study of 68 New World primates, different species of mycobacteria were detected by PCR in 65% of the primate population, of which 11% were diagnosed as *M. tuberculosis* by gene amplification and RFLP.[1] Only 54% of this population was culture positive.

Several immunoassays have been used for TB diagnosis in primates. The IFN-γ test (Primagam) uses whole blood and has been tested in gorillas, chimpanzees, orangutans, gibbons, colobids, baboons, mandrills, vervets, guenons, squirrel monkeys, langurs, and marmosets, but it cannot detect IFN produced by cells from *Macaca* spp.[4]

ELISA and MAPIA have also been used to evaluate serologic responses in nonhuman primates. *M. bovis*–infected macaques developed antibodies that were detectable in an ELISA using ESAT-6 as the antigen.[29] Although these tests are promising, they are not commercially available at this time.

Routine screening of primate collections depends on the history of the collection and assessment of risk factors, such as exposure to other primates, including humans. Because mycobacterial infections may be insidious, periodic screening is recommended even in closed collections. A thorough necropsy of every nonhuman primate that dies should be performed and mycobacterial culture and PCR of thoracic lymph nodes and other tissues considered even in the absence of gross lesions, if there has been a history of exposure or infection in the group. Tissue should be archived for future analysis if any suspicious lesions are observed.

Carnivores

In general, TB in carnivores occurs only sporadically from incidental infection through close contact with infected reservoir hosts or ingestion of infected animals. *M. bovis* has been detected in lions, cheetahs, domestic dogs and cats, leopards, tiger, red fox, and fennec fox, and *M. tuberculosis* complex in snow leopards and domestic dogs and cats.[2,25,27]

The intradermal skin test has been used to screen lions antemortem.[41] South African lions in an area with a high prevalence of *M. bovis* were tested using an intradermal CCT.[7] Positive skin tests showed good correlation with necropsies revealing suspicious lesions and positive cultures. Therefore, it appears that comparative intradermal testing may be modified for use as a screening test in lions and potentially other exotic felids.

Routine tuberculin testing of felids and canids is not standard in most zoologic collections. Imported or wild-caught carnivores from regions that have a known TB reservoir should be screened during quarantine. Additionally, carnivores that are fed carcasses that might harbor organisms (e.g., whole-prey feeding practices) should be evaluated periodically. The diagnostic workup includes CBC; thoracic radiographs; tracheal/gastric lavage, feces, or tissue for acid-fast stain; PCR; and mycobacterial culture. A single or comparative intradermal tuberculin test using bovine and avian PPD may also be used for screening, although response data are extremely limited for most carnivore species. PCR has been useful in rapid detection of organisms and distinguishing between *M. avium* and *M. tuberculosis* complex with appropriate primers. DNA fingerprinting is useful for identification of strains and epidemiologic investigation. A thorough necropsy should be performed on any carnivore that dies and tissues cultured and archived if there is a suspicion of TB.

Immunoassays have also been used to a limited degree in carnivores. Serum from a *M. bovis*–infected lion was positive in ELISA to *M. bovis* antigens, whereas tuberculin test–negative cage mates were ELISA negative.[41] ELISA has also been used to screen East African lions.[10] Recently, sera from a group of *M. bovis*–infected jaguars were tested using Rapid Test and MAPIA.[34] Serologic results were consistent with culture status. IFN-γ tests, similar to Primagam, have not been developed for carnivores to date.

Small Mammals

Tuberculosis has been diagnosed in ferrets, hedgehogs, badger, voles, hyrax, rabbit and hare, stoats *(Mustela erminea),* mole *(Talpa europaea),* and brown rat and reproduced experimentally in mice, rabbits, and guinea pigs.[15,18] The primary focus of testing has been identification of wildlife reservoirs for management and control. Most cases are diagnosed postmortem based on gross lesions, histopathology, culture, and PCR identification of the mycobacterial organism, usually *M. bovis.* Immunoassays detecting cell-mediated responses and antibody have been investigated in *M. bovis*–infected badgers.[23,44] Although not routinely screened, a case of TB caused by *M. microti* in an imported hyrax emphasizes the need for surveillance and the lack of available tests for TB detection in these species.[15]

The diagnostic workup for any suspect case includes CBC; thoracic or whole-body radiographs;

tracheal/gastric lavage, feces, or tissue for acid-fast stain and PCR/MTD; and mycobacterial culture with speciation. Intradermal tuberculin test has not been evaluated in the majority of these species. DNA fingerprinting should be performed when possible to determine relatedness of isolates and origin when more than one case is involved.

Marsupials

Mycobacterial infections are important diseases of marsupials, although *M. bovis* has been found primarily in the brushtail possum.[11] *M. avium* and other atypical mycobacteria are a greater concern for other marsupials, such as tree kangaroos and wallabies.[28] These infections usually present as osteomyelitis. *M. bovis* and *M. tuberculosis* may also cause osteomyelitis, so it is important to be able to distinguish between these infections.

Tuberculin testing of marsupials has not been standardized. It appears that differences in cell-mediated immune response may play a role in the preponderance of primarily *M. avium* infections observed in this group of mammals.[40] Positive intradermal tuberculin tests to *M. avium* have been observed in infected tree kangaroos.[28] Diagnostic examinations should include CBC, chemistry panel, whole-body radiographs that include the skeletal structures, acid-fast stain, mycobacterial culture, and PCR on exudates from draining tracts, lymph node, or other biopsy samples (bone). ELISAs were evaluated in possums but had insufficient sensitivity for widespread application in field situations.[6] Molecular techniques, such as PCR and DNA fingerprinting, may be used to distinguish among the various mycobacterial species, which is important from a regulatory, zoonotic disease potential, and disease management perspective.

Routine evaluation of marsupials for mycobacterial infection is not typically performed except in quarantine or wildlife screening programs. If marsupials are being examined for other reasons (e.g., routine or preshipment exam), an assessment to rule out asymptomatic infection should be included.

Marine Mammals

Marine mammals are susceptible to infection with a variety of mycobacterial species. Tuberculosis has been found in both captive and wild pinnipeds, caused by a unique member of the *M. tuberculosis* complex, *Mycobacterium pinnipedii*.[14] This organism is also pathogenic in guinea pigs, rabbits, humans, and Brazilian tapirs. Clinical signs include depression, lethargy, dyspnea, and weight loss. Asymptomatic infection and acute mortality may occur in affected populations.

Diagnosis of TB in pinnipeds usually includes CBC, chemistry panel, ELISA using mycobacterial antigens, thoracic radiographs, acid-fast stain, mycobacterial culture, and PCR of respiratory or other exudates/tissue, and intradermal tuberculin tests. Tuberculin tests using bovine and avian PPD have been assessed in several species of pinnipeds.[42] Of 40 animals tested, 14 reacted positively to both tuberculins. Ten (of 14) responders had gross lesions at necropsy and/or positive cultures. ELISA results using *M. bovis* antigen also appears to correlate with mycobacterial infection, although it is unknown how exposure to nontuberculous mycobacteria may affect results.[13]

Routine TB testing is not usually performed in pinnipeds. Because *M. pinnipedii* apparently may be brought into a collection with wild-caught animals, however, screening in quarantine and periodic opportunistic testing should be considered as part of the preventive veterinary medical program.

Ungulates (Bovids, Giraffe)

Tuberculosis in artiodactylids is usually caused by *M. bovis* but has also been associated with *M. tuberculosis* infections. Although the U.S. federal eradication program only requires testing of cattle, bison, and cervids, the disease is reportable in all species. The *caudal fold tuberculin test* (CFT) is the official test for routine use in cattle and bison. The CFT is performed by injecting 0.1 mL of bovine PPD tuberculin (1 mg/mL) intradermally in the tail skin fold, with reading by visual observation and palpation at 72 (±6) hours. The *comparative cervical tuberculin test* (CCT) is the official method for retesting suspects. The bovine IFN-γ assay may be used as an alternative method for retesting cattle herds, with appropriate approval (USDA APHIS Bovine TB Eradication Uniform Method & Rules, 2005). Histopathology, mycobacterial culture, and PCR are also approved supplemental diagnostic procedures.

Among exotic species, TB has been recorded in greater and lesser kudu, common duiker, African buffalo, lechwe, eland, impala, American bison, water buffalo, Arabian oryx, East African oryx *(Oryx gazelle beisa),* wildebeest, topi, bushbuck, goats, sheep,

mountain goat, addax, sable antelope, and giraffe, although all cloven-hoofed ungulates are considered susceptible.[2,10,11,16] Surveys of tuberculin testing in zoo hoofstock have indicated variability in types of tuberculin used, site of injection, and interpretation of tests.[45,50] The National Tuberculosis Working Group for Zoo and Wildlife Species has developed standardized recommendations for intradermal testing in exotic ungulates. For program species (bison, domestic cattle) and *Bos*, *Bubalus,* and *Snycerus* bovids, the recommended test site is the caudal tail fold. The *single cervical tuberculin test* (SCT) is recommended for all other exotic bovids using 0.1 mL of bovine PPD, read at 72 hours. TB testing in giraffe is usually performed by CFT or SCT. Unless there is a history of TB in the herd or suspicion of infection based on clinical signs, immobilization for routine screening of giraffe is not recommended.

Because of variable sensitivity and specificity of intradermal testing in exotic hoofstock, other diagnostic tests should also be used, especially if an animal has a suspected infection. ELISA has been used in a limited number of species and may aid diagnosis in anergic individuals.[16] Nasal swab, tracheal/bronchial lavage, or material from draining lymph nodes or other tissue may be sent to the laboratory for mycobacterial culture, acid-fast stain, and PCR/MTD.

Immunoassays have been adapted for use in exotic ungulates, but development is often hindered by the need to develop species-specific test parameters or reagents. The IFN-γ assay and LT test are both experimental and have been used in a limited number of exotic hoofstock species, such as American bison and African buffalo.[2,24] Rapid Test and MAPIA were positive in an *M. tuberculosis*–infected Addra gazelle.[34] It appears that serologic tests may be useful as ancillary tests in some species.

Because of the possibility of TB in exotic bovids and regulatory concerns, it is recommended that zoo ungulates undergo screening during quarantine. Frequency of routine testing of collection hoofstock will depend on relative risk and factors such as potential exposure to infected animals, both inside the collection and outside (i.e., wildlife reservoirs), herd history, management practices, and environment. Similar to the requirements for domestic cattle herd accreditation, after initial screening of the herd, it would be prudent to screen adult animals every 2 years, or as the opportunity arises, because immobilization or handling may not be warranted in some situations. All hoofstock that die or are euthanized should receive a complete necropsy, especially focusing on the cervical and thoracic lymph nodes and respiratory system, to rule out TB.

Cervids

Cervid TB is an important disease in captive and free-ranging populations worldwide. *M. bovis* has been found in a wide variety of species, including elk, white-tailed deer, sika deer, reindeer, mule deer, fallow deer, and moose, although *M. tuberculosis* and *M. avium* have also been isolated.[11] Because of potential zoonotic and agricultural impacts, cervid TB is a federally regulated program in the United States.[12] Interstate movement of cervids in the United States requires TB testing of the cervids. States may adopt more stringent requirements regarding intrastate movement. AZA-accredited facilities are exempt from some of the rules when moving cervids between member facilities. These regulations are subject to change and should be checked before transport.

Currently, the SCT is the primary diagnostic test used in captive cervid herds with animals older than 1 year (USDA APHIS Bovine TB Eradication UMR, 1999). The test is performed by intradermal injection of 0.1 mL of bovine PPD tuberculin (1 mg/mL) in the midcervical region, with reading by visual observation and palpation at 72 (±6) hours. The CCT is used for retesting SCT suspects and is administered by a state or federal veterinarian. Histopathology, mycobacterial culture, and PCR are supplemental diagnostic procedures approved in the federal program. Results of all approved tests must be submitted to state and federal animal health officials.

Because of variable specificity and sensitivity of these tests and the difficulty distinguishing *M. bovis* infections from those caused by *M. avium* and other mycobacteria, alternate diagnostic tests should also be performed in suspect cases.[12] The BTb test, a combination of ELISA and LT assay, is no longer available in the United States as a commercial assay but has been replaced with an IFN-γ assay, Cervigam. This may be used as an ancillary test to CCT. Other diagnostic tests include lymphocyte stimulation tests, ELISA, immunoblot, Rapid Test, and MAPIA.[11,12] These have been especially helpful in species such as reindeer in which the low prevalence of TB and high frequency of false-positive tuberculin reactions have led to difficulty with diagnosis.[49]

A sound preventive medicine program should include regular TB testing of cervids in the zoologic collection. Incoming cervids should be tested before

transport and/or before leaving quarantine by tuberculin skin test and at least one ancillary test method, if available; otherwise, serum should be banked. Frequency of routine screening of cervid herds will depend on herd and collection history of TB exposure, type of herd management (closed or regular new additions), exposure to other potential sources of infection (e.g., mixed-species exhibits), and risk of handling for testing. Because the federal program requires an accredited TB-free cervid herd to pass two repeat herd tests every 2 to 3 years, screening of zoo cervids at the same frequency would be reasonable, using a combination of SCT and available blood-based tests.

Any cervid showing clinical signs consistent with *M. bovis* infection should receive a thorough examination, including CBC, chemistry panel, thoracic radiographs, and SCT; tracheal/bronchial lavage for acid-fast stain, mycobacterial culture, and PCR/MTD; possible lymph node aspirate or biopsy for histopathology and culture, PCR, and acid-fast stain; and blood collected for immunoassays, if available (IFN-γ production, ELISA, Rapid Test, MAPIA, Ag85). Complete necropsy should be performed on a cervid that dies or is euthanized, with special emphasis on head, cervical, thoracic lymph nodes, and respiratory system.

Camelids

Tuberculosis is found in both New World and Old World camelids. Routine screening is recommended as part of their regular health evaluation and may be required by regulatory agencies for interstate or international movement. Intradermal testing is usually performed by clipping hair in the postaxillary region and injecting 0.1 mL (5000 tuberculin units) of bovine PPD tuberculin. Skin thickness is measured at injection and 72 hours later, and any increase greater than 1.0 mm is interpreted as a response (USDA APHIS VS National Center for Import and Export). Responders should be retested by CCT. Additional diagnostic testing may include thoracic radiographs in smaller individuals; mycobacterial culture, acid-fast stain, and PCR of tracheal/bronchial wash or other fluids/tissues; and immunoassays, if available. Although bacille Calmette-Guérin (BCG)–vaccinated alpacas showed some response in LT and ELISA, experimentally *M. bovis*–infected llamas did not demonstrate a positive serologic response.[26,47] In naturally infected Bactrian camels, ELISA and immunoelectrophoresis detected antibodies to multiple mycobacterial species, including *M. bovis,* which may explain why camelids show a high frequency of false-positive tuberculin reactions.[8] Rapid Test has shown promise in diagnosing naturally infected Old World camels.[34]

Camelids should be screened regularly for TB as part of a thorough preventive health program, including quarantine and preshipment evaluation. Frequency of screening can be determined based on ease of handling, history of the individual, herd, and collection.

Tapirs

Pulmonary infection with *M. bovis* and *M. tuberculosis* has been reported in captive tapirs.[45] Regular screening is recommended. Bovine PPD tuberculin (0.1 mL) should be injected in the inguinal region near the nipples, although the skin around the perineum may also be used. Similar to camelids, tapirs may show nonspecific reaction to intradermal testing, confounding interpretation. Another recommended method of diagnostic screening is to flush 20 mL of sterile saline in one nostril, then collecting the rinse by gravity or aspiration in a vial for mycobacterial culture and PCR.[43] Immunoassays developed for other species, such as LT and ELISA, have been evaluated on a limited basis in tapirs, but may not be available.

Rhinoceroses

Tuberculosis has been diagnosed in captive black and white rhinoceroses.[33,37,46] Both *M. tuberculosis* and *M. bovis* have been isolated from black rhinoceroses. Intradermal testing using 0.1 mL of bovine PPD injected in the eyelid, base of the ear, or caudal tail fold has been used for screening rhinoceroses.[22] If present, swelling should be followed by immobilization to collect tracheal lavage for acid-fast stain, mycobacterial culture, and PCR for identification.[33] Serologic tests, such as ELISA, Rapid Test, and MAPIA, are also being investigated in these species.

With the increased use of husbandry training and restraint chutes for rhinoceroses, health screening may be accomplished on a more regular basis. Tuberculin testing and serologic screening should be incorporated into the preventive health program for these species based on history of the herd and collection. All rhinoceroses should ideally be screened as part of a thorough preshipment and quarantine evaluation.

Elephants

See Chapter 43 for a discussion of tuberculosis in elephants.

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