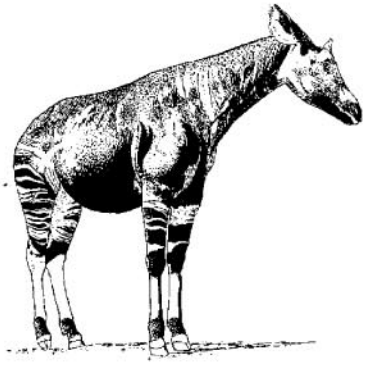
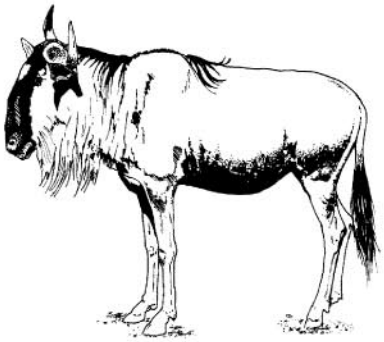
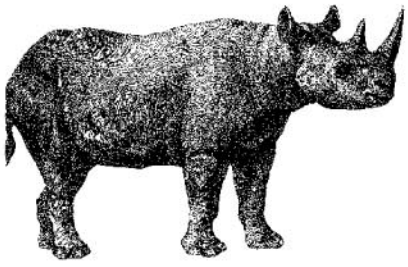
Tuberculosis Surveillance Plan for Non-Domestic Hoofstock







Developed by
The National Tuberculosis Working Group
for Zoo and Wildlife Species
October 2001

Endorsed by
American Association of Zoos and Aquariums
American Association of Zoo Veterinarians
USAHA Tuberculosis Committee
U.S. Department of Agriculture

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1 Introduction

The purpose of this surveillance plan is to:

1) Establish a standard for intradermal tuberculin testing of hoofstock species (other than domestic cattle, bison and deer) housed in zoological collections.
2) Collect prospective data on intradermal tuberculin testing and other diagnostic tests in order to determine sensitivity and specificity. Establish guidelines for the interpretation of results and for the course of action in the interim period before the skin test is validated or other methods of diagnosis are identified.
3) Accurately estimate the true prevalence and incidence of tuberculosis in zoological collections and assess the risk of transmission to program species.
4) Provide guidance for state and zoo veterinarians, curators, keepers and other staff exposed to tuberculosis-infected animals.
5) Prevent the transmission of TB within and between zoological collections.

The diagnosis of tuberculosis can have a devastating impact on a zoological collection. Animal movement may be restricted for years, issues of human health are often raised, and healthy animals may be euthanised.

Tuberculosis in ungulates has occurred in at least 20 accredited U.S. zoological collections in the past two decades. (Essey, pers comm). *Mycobacterium bovis* (the cattle strain) is generally the causative agent. *M. tuberculosis* (the human strain) may also, although rarely, cause disease in hoofstock. In the absence of any published guidelines, these situations have been dealt with in a variety of ways. Within the zoo community, both the American Zoo and Aquarium Association (AZA) and the American Association of Zoo Veterinarians (AAZV) recommend TB testing, however, no standard protocols are currently in place.

The intradermal tuberculin test is the primary screening test to detect TB in domestic cattle. Under the Cooperative State/Federal Tuberculosis Eradication Program, reactors are slaughtered. This program has proven effective to control TB in cattle in the United States. The intradermal tuberculin test has not been validated for species other than domestic cattle, bison, and cervidae. Numer-

ous reports in the literature suggest that the intradermal skin test may yield false positive results in non-domestic hoofstock. A false positive test may result in the unnecessary euthanasia of healthy animals; a false negative test may result in continued exposure of the zoo population and staff to the infected animal. While the test and slaughter method has been effective to control TB in domestic cattle, it may be inappropriate for ungulates in captivity because of their genetic value to the population.

It is essential that we begin to document and evaluate the results of available diagnostic tests so that future decisions regarding the diagnosis and treatment of this disease can be based on sound scientific evidence. There is a stigma associated with TB that must be overcome. This is not a disease to hide or to ignore. We can no longer avoid screening animals because test results are confusing and difficult to interpret.

Currently, federal regulations require tuberculin testing prior to inter-state shipment only for program species (domestic cattle, bison and cervidae). State requirements to permit movement of other non-program ungulates vary considerably. Also, most states have the authority to impose quarantine on a zoological facility and restrict animal movement out of the facility.

In 1996, the Tuberculosis Committee of the United States Animal Health Association (USAHA) recommended that "…the Animal and Plant Health Inspection Service (APHIS), Veterinary Services, pursue the formation of an inter-industry working group to address the issues of tuberculosis in exotic animal collections, including, but not limited to zoos, menageries, circuses, and other single or multiple species collections."

In 1998, this committee drafted an Emergency Plan for the Rapid Eradication of Bovine Tuberculosis from the United States (One Hundred and Second USAHA Annual Meeting, 1998, pg. 715-725) to support the goal of the Cooperative State/Federal Tuberculosis Eradication Program to eradicate bovine tuberculosis by December 31, 2002. The following excerpts from this Emergency Plan pertain to the zoo community:

- Bovine tuberculosis exists in zoological collections, and while transmission to livestock has not been demonstrated from these sources, this threat remains. Transmission of bovine TB from menageries (i.e., unregulated exotic collections) to livestock has occurred.
- APHIS and states should take necessary steps to address TB in all susceptible animal species and settings where livestock exposure is a risk. Identify agencies and groups with management authority over species not covered by USDA authority and develop cooperative efforts to address TB via cooperative agreements and memoranda of understanding. Establish communications and cooperation with the National Tuberculosis Working Group for Zoo and Wildlife Species.
- The Uniform Methods and Rules (UM&R) should be amended to clearly define standards for state status based upon risk. Standards need to be enforced. Standards should include risks of transmission from "covered" species, from free-ranging wildlife, and from zoological species including AZA zoos, non-AZA zoos, menageries, and other collections.
- In order to develop accountability and enhance epidemiological follow-up, any TB susceptible animal shipped from a zoo, menagerie or animal collection to any location other than an AZA accredited zoo must have a permit from the animal health official in the state of origin, prior to movement. Any such animals moving interstate must also have a permit from the state animal health official in the state of designation.

- Acquire the ability to prevent or control movement of all susceptible animal species that, due to their history or disease status, pose a risk to any domestic livestock, i.e., develop cooperative agreements and MOUs.
- Establish a task force by October 1999 to work with zoological and wildlife groups and agencies to develop surveillance and eradication procedures. Include indemnification or other monetary mitigation for other TB susceptible species.
- Work with the National Tuberculosis Working Group for Zoo and Wildlife Species to establish testing and movement protocols for zoological species.

The National Tuberculosis Working Group for Zoo and Wildlife Species was formed to address the concerns of the USAHA Tuberculosis Committee and the USDA regarding the occurrence of bovine tuberculosis in zoological collections and the potential risk of transmission to domestic livestock. The stated mission of the group is "to control and ultimately eradicate tuberculosis (*M. tb* complex) and control other mycobacterial diseases in zoo and wildlife species."

The diagnosis of *M. tuberculosis* in elephants in 1996 raised public concern for elephant and human health. In response, the USDA and the National Tuberculosis Working Group for Zoo and Wildlife Species developed a surveillance plan for this species. Guidelines for the Control of Tuberculosis in Elephants (www.aphis.usda.gov/ac) were distributed in January 1998. These guidelines utilize culture as the definitive method to diagnose TB in elephants, as other tests have not been validated for this species.

It is essential that a similar surveillance system be instituted for other ungulates in zoological collections. A test and slaughter program is not acceptable for endangered or valuable animals. Unless we immediately initiate a data-gathering system and pursue diagnostic alternatives to the skin test, we may have no alternative. The participation of the entire zoological community is essential to the success of this endeavor.

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2 Definitions

Tuberculosis Organism Terms

***Mycobacterium*:** A genus bacteria in the family Mycobacteriaceae.

***Mycobacterium tuberculosis (M. tb)*:** The primary causative agent of tuberculosis in humans; may also affect a variety of mammals, including non-human primates, pigs, cattle, dogs, parrots, elephants, and rhinos.

***Mycobacterium bovis (M. bovis)*:** The primary causative agent of tuberculosis in cattle, bison and cervids; may also affect a variety of mammals including pigs, humans, primates, and non-domestic ungulates.

***Mycobacterium tuberculosis* complex (MTB Complex):** A group of mycobacteria which includes *M. tuberculosis, M. bovis, M. africanum,* and *M. microti.*

***Mycobacterium avium* complex (MAC):** A group of mycobacteria which includes *M. avium avium*, *M. (?) intracellulare*, and *M. avium paratuberculosis*.

Disease Management Terms

At-risk person: Any person who has had direct contact with an infected animal(s) or who handles waste or other biological materials collected from an enclosure in which such animal(s) resides.

Exposed Non-Domestic Hoofstock: An animal that is known to have had, or may have had, nose-to-nose contact with the index animal or with infective biological material.

Exposure groups:

- **Primary exposure group:** TMU in which the index animal resides.
- **Secondary exposure group:** Animals exposed to animals in the primary group but not exposed to the index case.
- **Tertiary exposure group:** Animals with no known direct or indirect exposure to the index animal.

Fomite: An inanimate object or material on which disease-producing agents may be conveyed.

Herd: A group or groups of animals, maintained on common ground or two or more groups of animals under common ownership or supervision that are geographically separated, but that may have an interchange or movement of animals or personnel without regard to health status.

Incidence: The rate at which a certain event occurs, for example, the number of new cases of a specific disease occurring during a certain period.

Index animal: The animal in which the disease is first diagnosed.

Premises: A parcel of land containing animals, administered by a person, government entity (city, county, state, region), or organization (zoological society, corporation). A group of animals (one or more) maintained on common ground or two or more groups of animals under common ownership or supervision that are geographically separated, but may have an interchange or movement of animals without regard to health status.

Prevalence: The total number of cases of a specific disease in a given population at a given time.

Risk Status for TMU:

Low Risk: 1) TB never isolated in the collection, or
2) TB isolated > 5 years ago and all animals in primary and secondary exposure groups test negative (using recommended testing as documented in this plan) and routine testing plan in place.

Moderate Risk: 1) TB isolated > 5 years ago and all animals in TMU not tested (or test suspect) and no routine testing in place, or
2) TB isolated 0-5 years ago and all animals in primary and secondary exposure groups test negative (using recommended testing as documented in this plan) and routine testing program in place.

High Risk: 1) TB isolated 0-5 years ago and positives (or suspects) in primary and secondary exposure groups, or
2) TB isolated 0-5 years ago and primary and secondary exposure groups not tested and no routine testing in place.

Unknown Risk: 1) Not all animals tested, and
2) No routine testing plan in place.

Tuberculosis infection: When one or more animals are identified as being infected with *M. bovis* or *M. tb* by positive cultures from sputum, feces or other samples (e.g., tracheal wash), antemortem or from lesions obtained at necropsy.

Tuberculosis Infection Management Team: The group of individuals responsible for investigating and monitoring the tuberculosis status of an institution in which TB has occurred. The team may include the facility manager, the state USDA epidemiologist, the attending veterinarian, and other appropriate staff.

Tuberculosis Management Unit (TMU): The animal, species, enclosure, geographic area, or institution of concern.

Terms Pertaining to USDA Programs and Tests

(from Bovine Tuberculosis Eradication Uniform Methods and Rules)

Caudal-fold tuberculin (CFT) test: The intradermal injection of 0.1 ml of purified protein derivative (PPD) tuberculin (1 mg/ml PPD) into either side of the caudal fold with reading by visual observation and palpation 72 hours (plus or minus 6 hours) following injection. Cattle, bison, or goats will not be subjected to CFT retest at intervals of less than 60 days.

Cervical tuberculin (CT) test: The intradermal injection of 0.1 ml of USDA bovine cervical PPD tuberculin (2 mg/ml PPD) in the cervical region with reading by visual observation and palpation 72 hours (plus or minus 6 hours) following injection. Results of the CT test can only be classified as reactor or negative.

Comparative cervical tuberculin (CCT) test: The intradermal injection of biologically balanced USDA bovine PPD tuberculin and avian PPD tuberculin at separate sites in the midcervical area to determine the probable presence of bovine tuberculosis (*M. bovis*) by comparing the response of the two tuberculins at 72 hours (plus or minus 6 hours) following injection. For program species, the test shall be administered only by an approved state or federal veterinarian; for non-program species, the test may be administered by a state veterinarian, a federal veterinarian, or a zoo veterinarian that has completed a USDA-taught, AAZV-approved training program.

Cooperative State/Federal Tuberculosis Eradication Program: The bovine eradication program was initiated in 1917 in response to the public health risk posed by *M. bovis* in the nation's milk supply and economic losses associated with the disease in livestock. The prevalence of bovine tuberculosis in cattle has steadily declined from an estimated 5 percent early in the program to the current estimate of 0.0002 percent (2 cases in every 1,000,000 cattle). A goal of total eradication of bovine tuberculosis has been set for December 31, 2002. Bison were added as a "program species" in 1984, and cervidae were added in 1994. Detection of tuberculosis in free-ranging cervids and an unknown incidence of TB in zoological collections are considered to be obstacles to achieving the goal of eradication.

Designated accredited veterinarian: An accredited veterinarian trained and approved to conduct tuberculosis program activities.

Designated Tuberculosis Epidemiologist (DTE): A state or federal epidemiologist designated by APHIS to make decisions concerning the use and interpretation of diagnostic tests and the management of affected herds under this subpart who has demonstrated the knowledge and ability to perform the functions specified by the Bovine Tuberculosis Eradication UMR. The DTE must be selected jointly by the cooperating state animal health official, the area veterinarian-in-charge, and the regional epidemiologist. The National Animal Health Programs staff of VS must concur in the appointment. The DTE has the responsibility to determine the scope of epidemiologic investigations, determine the status of herds, assist in development of individual herd plans, and coordinate disease surveillance and eradication programs within his or her geographic responsibility. The DTE has the authority to make independent decisions concerning the use and interpretation of diagnostic tests and management of affected herds when those actions are supported by sound disease eradication principles.

Exposed animals: Any cattle, bison, captive cervids, goats, swine, or other livestock that have been exposed to bovine tuberculosis by reason of associating with other livestock from which *M. bovis* has been isolated.

Geographic separation: Geographic separation means a minimum of 30 feet of separation, no common or shared handling facilities and equipment, no common watering or feeding equipment, and no common feed vehicles entering the premises of herds of different statuses. Also, if the herds are fed by the same personnel, workers must wear different outerwear (e.g., boots and coveralls) for each herd.

Herd: Any group of livestock maintained on common ground or two or more groups of livestock under common ownership or supervision that are geographically separated from other herds but can have an interchange or movement without regard to health status, as determined by the APHIS administrator. A group means one or more animals

Individual herd plan: A written disease-management plan that is designed by the herd owner, other herd representative, or both, and a state or federal veterinarian to eradicate tuberculosis from an affected herd while reducing human exposure to the disease. The herd plan will include appropriate herd test frequencies, tests to be employed. And any additional disease-management or herd-management practices deemed necessary to eradicate tuberculosis from

the herd in an efficient and effective manner. The plan must be approved by the state animal health official and the area veterinarian-in-charge and have the concurrence of the regional or designated tuberculosis epidemiologist.

Livestock: Cattle, bison, cervids, swine, dairy goats, and other hoofed animals (such as llamas, alpacas, and antelope) raised or maintained in captivity for the production of meat, and other products, for sport, or for exhibition.

Negative animals: Any cattle, bison, captive cervids, or goats that show response to the tuberculin test, are classified by the testing laboratory as "avian" or "negative" on the BTB test, or are classified negative for tuberculosis by the testing veterinarian based upon history, supplemental tests, examination of the carcass, and histopathology and culture of selected tissues.

Official tuberculin test: A test for bovine tuberculosis, approved by APHIS, applied and reported by approved personnel in accordance with the UMR. The official tuberculin tests for cattle, bison, and goats are the caudal-fold test, the comparative cervical test, and the cervical test.

Official tuberculosis test (captive cervid): A test for bovine tuberculosis applied and reported by approved personnel in accordance with these UMR. The official tests for captive cervids are the single cervical test, the comparative cervical test, and the blood tuberculosis test.

Reactor: Any bovid (genus *Bos*), captive cervid, bison, or goat that shows a response to an official tuberculosis test and is classified a reactor by the testing veterinarian or DTE, or any suspect animal that is classified a reactor upon slaughter inspection or necropsy after histopathology and/or culture of selected tissues by the USDA or state veterinarian performing or supervising the slaughter inspection or necropsy.

Single cervical tuberculin (SCT) test (captive cervid): The intradermal injection of 0.1 ml of USDA bovine PPD tuberculin (1 mg/ml PPD) in the midcervical region with reading by visual observation and palpation 72 hours (plus or minus 6 hours) following injection. This test shall be administered only by a state, federal, or designated accredited veterinarian. Captive cervids will not be subjected to CFT retest at intervals of less than 90 days.

Suspect: 1) Any cattle, bison, or goat that shows a response to the caudal-fold tuberculin test and is not classified a reactor; or cattle, bison, or goats that have been classified suspects by a comparative cervical test. 2) Any captive cervid that is not negative to the SCT test or the CCT test, or that is classified by the testing laboratory as equivocal in response to the BTB test, and that is not classified as a reactor by the testing veterinarian.

Tuberculin: A product that is approved by, and produced under, USDA license for injection into livestock for the purpose of detecting bovine tuberculosis.

Uniform Methods and Rules (UM&R): A set of standards, developed by state and federal regulatory officials as well as industry and academia representatives, which details the minimum requirements necessary to have state disease control programs that are consistent nationwide.

Terms for Culture and Ancillary Diagnostic Tests

Culture test positive: Isolation and identification of *M. tb* or *M. bovis* from any site using standard mycobacterial methods.

ELISA: Enzyme-linked immunosorbent assay; a test used to detect and measure either antigen or antibody.

Gamma-interferon test: A whole blood *in vitro* assay currently being evaluated as a diagnostic test for TB.

Mycobacterium Tuberculosis Direct Test (MTD): A nucleic acid amplification test used in the diagnosis of TB. The MTD utilizes a technique that replicates RNA from bacteria of the *M. tuberculosis* complex.

No isolation: No growth of *M. tb* or *M. bovis* from respiratory wash, feces, tissue or other sample using approved mycobacteria culture methods. **Does not rule out the possibility of *M. tb* or *M. bovis* infection.** Possible explanations for no isolation are:

1. Non-infected animal
2. Infected animal but not shedding organisms at the time of testing (may indicate early infection or latent disease)
3. Sampling error (culture overgrowth by contaminating organisms, inadequate sample), laboratory error, or no isolation
4. Effectively treated animal

Nucleic acid amplification test: A technique that amplifies entities such as DNA or RNA.

PCR (polymerase-chain reaction): a nucleic acid amplification technique in which specific sequences of DNA are replicated, allowing for detection of target sequences that otherwise would not be present in high enough numbers to be detected.

Sensitivity: A measure of the ability of a test to identify infected animals.

Specificity: A measure of the ability of a test to identify non-infected animals.

3 General Preventive Measures

A number of procedures can and should be instituted to prevent the transmission of TB between institutions:

- Identify the TB status of the sending institution (see risk status group definitions pg. 6)
- Avoid animal exchanges with facilities that do not have a TB surveillance plan in place.
- Maintain farm animals separate from non-domestic ungulates in the collection. The transfer of domestic livestock from zoos to farms should be avoided when possible to minimize the potential risk of transmission from the zoo to the livestock industry. If such transfers occur, appropriate preshipment testing should be conducted.
- An institutional surveillance plan should be developed and instituted. In addition to protocols for testing ungulates, it should include protocols for screening animal personnel. Pre-employment and annual tuberculin testing are recommended.
- Screen hoofstock before shipment. All animals that are shipped should be tuberculin tested on the premises of the sending institution as part of a pre-shipment physical. **Tuberculin tests should be read by palpation**. Visual interpretation is not acceptable. A negative test does not guarantee that the animal is tuberculosis-free and a positive test does not necessarily mean that the animal is infected. Animals that respond to intradermal test should be further evaluated by culture and supplementary diagnostic tests described on page 19. Test results should be interpreted taking into consideration the history of the individual animal enclosure, the previous movement history of the animal and the TB risk status of the institution. *The overall status of the institution or TMU is more important than one test result from an individual animal.* All animals should have a permanent identification (transponder or tattoo).
- Ungulates should be quarantined upon arrival according to guidelines established by AZA (Quarantine Procedures Recommended for AZA Accredited Institutions, 1991) and AAZV (Preventive Medicine Recommendations, Infectious Disease Reviews, 1995). Animals not screened for TB prior to shipment should be tested during quarantine at the receiving institution.

4 Surveillance Plans

General Surveillance

Routine surveillance by the entire zoo community is essential for the control of tuberculosis. Cooperation in collecting and reporting data is critical to improved diagnostic and management techniques. In addition to testing animals prior to shipment, hoofstock that are part of the collection should be evaluated when they are immobilized for other procedures. This evaluation should include an intradermal tuberculin test and other diagnostic tests as described in Ch. 5—Diagnostic Testing and Data Collection. The intradermal skin test requires direct measurement of the test sites at 72 hours, plus or minus 6 hours. Unless animals can be managed in a chute, this will require two immobilizations. Individual animals should be tested annually if possible. Clinical signs consistent with mycobacteriosis, such as chronic weight loss, cachexia, and/or poor condition, should be isolated and evaluated as quickly as possible. Necropsies should be performed on all ungulates that die. In addition to gross examination, it is recommended that tissues be submitted for histopathology. Any lesions that are suspicious for tuberculosis should be submitted for culture. Refer to App. 3 Ungulate Necropsy Protocol (General) and App. 5 Ungulate Necropsy Protocol (TB Suspects).

Surveillance Plan for Unknown Risk TMU

Definition

1) Not all animals tested, and
2) No routine testing plan in place

Plan

- All non-domestic hoofstock should be tested within a 30 day period using recommended testing methods as described in these guidelines.
- A TB testing plan should be developed based upon initial testing results.

Surveillance Plan for Low Risk TMU

Definition

1) TB never isolated in collection, or
2) TB isolated > 5 years ago and all animals in primary and secondary exposure groups test negative (using recommended testing as documented in this plan) and routine testing plan in place.

Plan

- All non-domestic hoofstock should be tested during immobilization using recommended testing methods as described in these guidelines, but tests should not be performed more than once per year.
- All non-domestic hoofstock should be tested prior to being transferred, relocated, or otherwise moved from the TMU.

Surveillance Plan for Moderate Risk TMU

Definition

1) TB isolated > 5 years ago and all animals in TMU not tested (or test suspect) and no routine testing in place, or
2) TB isolated 0–5 years ago and all animals in primary and secondary exposure groups test negative (using recommended testing as documented in this plan) and routine testing program in place.

Plan

- All non-domestic hoofstock should be tested yearly (using recommended testing methods as described in these guidelines) until no reactor animals are found for five years. All suspect animals should receive a secondary TB test (as described in these guidelines).
- All non-domestic hoofstock should be tested prior to being transferred, relocated, or otherwise moved from the TMU.

Surveillance Plan for High Risk TMU

Definition

1) TB isolated 0–5 years ago and suspects in primary and secondary exposure groups, or
2) TB isolated 0–5 years ago and primary and secondary exposure groups not tested and no routine testing in place.

Plan

- *Epidemiology and Movement Restrictions*
  All mammals residing on the premises where tuberculosis has been confirmed by isolation of the causative organism, should initially be quarantined until a thorough epidemiological study can be conducted to determine which animals are at significant risk of infection.

Epidemiology should include trace-outs of all confirmed infected and exposed animals and notification of all facilities that may have received such animals of their possible exposure to tuberculosis. Additionally, the investigation should include an effort to locate the source of the infection by traceback and investigation of all outside acquisitions. The TB Management Team (consisting of the facility manager, the state USDA epidemiologist, the attending veterinarian and other personnel deemed appropriate) may then decide which animals should be tested and quarantined and which animals, if any, may be released at that time. The TB Management Team should also determine the boundaries of the TB Management Unit for purposes of subsequent testing to focus the investigation.

- *Testing*

  The TB Management Team should determine which animals should be tested, the frequency of that testing, and the specific testing procedures that will be used in addition to intradermal tuberculin testing (chest radiographs, serology, tracheal wash and culture, etc.). Ancillary tests are highly recommended for high risk TMUs. False positive and false negative intradermal skin test results occur frequently in non-domestic species. All species on the premises covered by USDA, Veterinary Services (cattle, bison, and cervidae) should be tested according to established rules, regardless of exposure status. Non-mammalian species housed in direct contact with confirmed cases should be considered.

  In make testing decisions; consideration should be given to the relative risk associated with testing (i.e., chemical immobilization or restraint that may result in injury or death) versus the potential value of the test result. Given the knowledge acquired from previous work with domestic species, it is recommended that the testing regimen for release from quarantine be comparable to that used for cattle or cervidae (i.e., two negative herd tests 60-90 days apart followed by another negative test 180 days later) until more research can be done on testing and infection risks in wild/exotic species. Consideration should be given to dividing the TB Management Unit into primary, secondary, and tertiary risk groups (see Definitions, Ch. 2).

- *Disposition of Infected Animals*

  Treatment. While treatment is not encouraged, it may be considered an option in certain situations (endangered species or other valuable animals). Treatment may require long term observation and surveillance.

  Research and Data Collection. Information from moderate to high-risk TB management units is particularly valuable. Institutions in this category should make every effort to test animals in their collections, submit samples for ancillary tests, and keep accurate records.

  Other. Consideration may be given to using the herd for producing offspring or for assisted reproduction.

  Euthanasia. Euthanasia followed by a complete necropsy with laboratory follow-up should be seriously considered for infected animals. [Refer to Ungulate Necropsy Protocol (TB Suspects), App. 5]. It is strongly recommended that a complete diagnostic work-up be conducted on all animals prior to euthanasia and that samples be submitted for all primary and ancillary tests. Information from known positive animals is essential to the validation of pre-mortem diagnostic tests.

- *Disposition of Exposed Animals*

  Isolation. Exposed animals should be housed in isolation from other animals in the collection. Testing and monitoring should be carried out according to protocols described

elsewhere in this document. Although euthanasia may be considered for exposed animals, these animals are important research subjects to further our knowledge of this disease. Much information can be learned by monitoring these animals over time. Animals that are euthanized should undergo a complete necropsy.

Treatment. Prophylactic treatment may be considered in endangered species or extremely valuable animals, but only after the initial testing procedure is completed.

- *Premises Disinfection*

  Any enclosure housing an infected animal should be thoroughly cleaned and disinfected using an approved anti-mycobacterial disinfecting agent. All hard surfaces and feed and water receptacles should be cleaned and disinfected. Consideration should be given to potential toxicity in susceptible species (i.e., felids). When feasible, the top 6–8 inches of soil should be removed, disposed of in a sanitary landfill (or in compliance with local hazardous waste disposal regulations) and replaced with fresh dirt. Premises that have been disinfected following removal of an infected animal should remain empty for at least 30 days.

- *Public Relations*

  The facility should be pro-active in addressing tuberculosis. Press releases should be prepared when appropriate and each facility should develop a standard operating procedure to handle a confirmed infection. The facility spokesperson should be part of the TB Management Team or should communicate directly with the Team. Professional public relations personnel are recommended to handle media inquiries. The development of fact sheets for release to the public may be helpful. Guidelines should be widely distributed, not only to animal owners and zoos, but also to state veterinarians, the Centers for Disease Control and Prevention (who may then distribute them to other public health officials), and to animal interest groups.

- *Staff Relations*

  Open communication with facility staff is essential, particularly when TB has occurred. Meetings with facility staff and outside professionals to provide accurate information about TB should alleviate concerns, dispel misconceptions, and communicate to staff that human and animal health issues are being addressed in a professional manner.

- *Human Health*

  All at-risk persons should be tested for tuberculosis following professionally accepted medical practices. (Refer to Zoo Personnel Health Program Recommendations in *Infectious Disease Reviews,* AAZV, 1995).

- *Regulatory Notification*

  In confirmed outbreaks, the following persons or agencies should be promptly notified.

  1. State veterinarian
  2. USDA, Veterinary Services
  3. USDA, Animal Care (for facilities licensed under the Animal Welfare Act)
  4. Public health officials
  5. Other state or local regulatory agencies as appropriate

5 Diagnostic Testing and Data Collection

A three-year prospective study using the following protocols has been developed to collect and tabulate data related to tuberculosis diagnostics in nondomestic ungulates in a uniform manner. Three separate methods—MedARKS, web-based, and paper-based—are available for recording and compiling data (see Data Collection and Reporting, pg. 20).

Skin Testing Recommendations

These sites were selected with ease of testing and potential biopsy of the tuberculin site in mind. These tests should not be performed on animals under 6 months of age.

- The initial screening test for tuberculosis diagnosis in nondomestic hoofstock is the **single intradermal test** utilizing 0.1 ml contract PPD bovis. The test site should be checked by palpation at 72 hours (plus or minus 6 hrs.) in most species. Swine should be checked at 48 hours (plus or minus 6 hrs.).
- When this test is suspect, it is recommended to apply the **comparative tuberculin test** using 0.1 ml of balanced PPD avian and bovis tuberculin each administered at a separate site. A hands-length should separate the two test sites on the same side of the animal. The tuberculins should be placed on opposing sides in situations where the sites cannot be separated by a hands length, where separation forces injection of tuberculins into skin of different thicknesses differences, or when stated to do so below.

  This test can be performed within 10 days of an initial intradermal tuberculin test or 60 days after the last intradermal test. The exception to this is in cervids, which must wait 90 days post-intradermal testing. The side of the animal opposite from the single tuberculin test should be used for the comparative test if it will be performed within 10 days of the first intradermal test. It is very important not to perform the comparative test in the same site as the single tuberculin test. The comparative test should be checked in 72 hours (plus or minus 6 hrs) in most species. The site should be checked at 48 hours (plus or minus 6 hrs.) in swine. Tuberculins should be obtained through your state veterinary fficial. If this is not possible, then National Veterinary Services Laboratory should be contacted (App. 6). These tuberculins can only be obtained by appropriately trained zoo veterinarians or appropriate state of federal veterinary officers. If a veterinarian has not undergone the appropriate training needed to perform the comparative test, a request should be made to the state veterinary officer.

- The skin reaction can be biopsied on all responders. A **punch biopsy** works well for this purpose. A control site biopsy is not necessary. Send samples to an appropriate laboratory for histopathology to look for delayed type hypersensitivity reactions. Submit a good history with the sample. For more information, contact Dr. Richard Montali (App. 6).

Culture

Samples for tuberculosis culture should preferably be sent to USDA-National Veterinary Service Laboratory (see App. 6). Please send samples using the USDA VS Form 10-4 laboratory submission forms (App. 2) or samples will not be processed. Tracheal wash samples should be sent frozen, in leak-proof containers that can be supplied by NVSL. Twenty to 30 ml is preferred but not essential. The samples can be frozen and then sent via overnight mail on ice packs. Tissue samples should be 4-6 cm in size and frozen immediately. Send samples as soon as

Recommended Skin Testing Sites

Animal group	*Site*
Bovidae	Caudal fold in program species (bison, domestic cattle) and Bos, Bubalus and Snycerus-genus bovids, cervical test in all other bovids; cervical for comparative tuberculin testing
Giraffidae	Caudal fold is the preferred site for single intradermal tuberculin testing, behind the ear is an alternative site. For comparative testing the recommended site is the skin on the lateral surface of the neck 6-10 inches distal to the ramus of the mandible on one side
Cervidae	Lateral mid-cervical
Camelids	Hairless area caudal to the elbow in New World species. No test site is currently recommended for Old World species pending survey results
Other nondomestic artiodactylids	Lateral mid-cervical
Rhinos	Behind ear is the preferred site for single and comparative intradermal testing
Hippos	The equivalent of the caudal fold is the preferred site for single tuberculin testing, an alternative site would be behind the ear, which would also be used for comparative testing
Elephants	Tuberculin test not recommended. Refer to elephant guidelines
Tapir	Inguinal for both single and comparative testing
Swine	Fold at base of the ear for single tuberculin testing, caudal surface of the ear. Use the same site on both ears for comparative testing

possible after collection (no longer than one week). Do not use sodium borate with any sample. Cost: no charge. If an alternative laboratory is used, please forward the results to Dr. Payeur. The responsibility of forwarding these results lies with the clinical veterinarian. If outside laboratories have an isolate that cannot be identified, then the isolate should be forwarded on a slant agar media to NVSL (also the responsibility of the clinical veterinarian).

Mycobacterial culture of samples via tracheal wash is the current "gold standard" and is recommended in all suspect cases. Methods of sample collection may include direct tracheal culture, thoracoscopy using a rigid or flexible endoscope, endoscopy, bronchoscopy, thoracotomy, and/or laparoscopy. Acid-fast staining should be performed on the sediment of all samples collected (request this on submission form).

NVSL will be willing to send all positive culture samples to an outside lab for **drug sensitivity testing**. The clinician sending the sample must inform NVSL to which lab the sample should be sent. The submitting clinician's institution will be responsible for payment for drug sensitivity testing.

DNA Fingerprinting is performed on all positive cultures. It is used as an epidemiological tool. It is also performed at NVSL.

Other Diagnostics

a) ELISA

This diagnostic test can be conducted by either Dr. Thoen at Iowa State University or Dr. Salman at Colorado State University (App. 6). Send 2–4 ml of frozen serum for each test overnight on ice packs. At minimum, serum should be banked for this purpose. Sample storage at -70 degrees F is preferred for serum banking. Samples from known positive and known negative animals are needed. Blood collected from deceased animals that have undergone complete necropsies (including mycobacterial culture of lung and lymph nodes) are particularly valuable regardless of final TB status. Blood collected post-mortem soon after the animal has dies is generally acceptable if serum is promptly collected and frozen. Please use the appropriate form (App. 1) when submitting samples.

b) Nasal Wash or Swab for PCR

Collect saline or swab sample. Can be frozen in conventional freezer prior to shipment. Send overnight on ice packs to Texas A&M University (App. 6).

c) Chest Radiographs

Chest radiographs should be performed, where practical, on animals that are suspects for tuberculosis.

d) Nucleic Acid Amplification Techniques (PCR, MTD)

This technique can be used on diagnostic specimens such as tracheal washes and tissue samples. The advantage of this technology is that there is a rapid turn around time, and recovery rate is better than culture for samples contaminated with other bacteria. This technology should

be utilized in conjunction with culture. The test must be requested when submitting samples to NVSL for culture.

Polymerase chain reaction (PCR) is a series of reactions that results in the replication of specific sequences of DNA. Replication of DNA by PCR allows for detection of target sequences that otherwise would not be present in high enough numbers to be detected. PCR also allows for detection of specific target sequences because the primers will bind only to DNA that has a matching sequence.

A positive PCR test result means that DNA from *M. tuberculosis* complex organisms is present in the sample. PCR will detect both live and dead organisms. One advantage of the PCR test is that it is rapid. Results are usually available within one week of submission compared to a possible eight weeks required for cultures. Until further evaluation of the validity of the PCR/MTD test in nondomestic hoofstock, culture results should still be used as the definitive diagnostic test.

Send samples to NVSL (App. 6) or other suitable laboratory. Call ahead to ensure test availability. Liquid samples should be placed in 20-ml sterile tubes and shipped overnight on dry ice. Cost: approximately $30.

e) Gamma Interferon

This test is still in the developmental stage. Samples should be sent to USDA, ARS (App. 6) on suspect and known positive animals. It is essential to contact the lab before sending the sample. The sample must arrive within 16 hrs of collection. Results may not be available for some time. Send one 10-ml heparin tube at room temperature by overnight mail. Pack in a plastic foam container to protect from temperature extremes during shipment. Do not use cold packs or ice. No charge at this time.

Data Collection and Reporting

To better understand the epidemiology of tuberculosis infections in hoofstock and to develop better diagnostic tools to detect such infections, it is critical to collect and analyze disease prevalence and testing data from all zoological institutions. At the same time, security of these data must remain high to avoid misinterpretation of results. Therefore, the Working Group (under approval and guidance from USDA, AAZV and AZA) has developed a multi-level data collection plan focused on improving the methods to centralize these data for an initial three-year trial period. This plan includes:

1) ***Surveys***
   Through the formulation and implementation of directed questionnaires aimed at understanding the apparent prevalence of disease and testing practices in hoofstock species previously and currently being held at institutions, better understanding of this disease and those testing methods which work best in each species may be acquired. Only through a high compliance with these requests can appropriate conclusions be drawn to understand this disease, attempt to control disease spread and to serve the zoological community as a whole.
2) ***Prospective Testing Data Collection***
   All USDA-licensed facilities housing non-domestic hoofstock will be strongly encouraged

to provide tuberculosis testing data (both positive and negative results) to the Zoo and Wildlife Medical Databank (ZWMD) housed at Lincoln Park Zoo. To assist in this reporting and to make it as efficient as possible for contributing institutions, a three-phased approach will be developed:

a. **MedARKS Reporting—**For those institutions currently using MedARKS as their medical records system, a reporting method will be developed by ISIS such that information can be directly extracted from that currently being entered in an institution's database. This method will require a quarterly download (via e-mail) directly to the ZWMD using a simple one-step module entry and will be coupled with a brief training as to how to appropriately enter testing data into the database.
b. **Web-based Reporting—**For those institutions not using MedARKS for medical records storage but have appropriate computer and Internet access, a Web-based form for reporting test results will be developed. In order to ensure privacy of reporting, a username and password will be selected by the institutional reporting person at those institutions choosing this option, and data can be submitted by simply filling out an on-line Web form. Information will be encoded into the ZWMD using an institutional code to ensure data privacy.
c. **Paper-Based Reporting—**For those institutions not using MedARKS, with no Internet access, or preferring not to utilize these two avenues, a paper-based reporting form is available (App. 7). Forms will be mailed to the ZWMD where data will be encoded into the ZWMD using institutional codes to maintain security of information.

The information collected by this plan will be summarized at least annually and be provided to all participating institutions. These results will include overall prevalence statistics (exclusive of specific location information), incidence rate estimations, and improved guidelines for testing practices, methods, and interpretation. In addition, significant amounts of data will be available in the ZWMD on tuberculosis within the zoological community such that infectious disease researchers and diagnosticians can develop more in-depth projects. All requests for use of these data will undergo an approval process within the Working Group and data security will be maintained by the use of assigned usernames and close oversight by the Working Group.

Appendix 1. TB ELISA Sample Submission Form

TB ELISA Sample Submission Form for Non-Domestic Hoofstock	**Page _____ of _____**
Mycobacterium bovis Testing Laboratory Center of Veterinary Epidemiology and Animal Disease Surveillance Systems Colorado State University Environmental Health Bldg, RM 107 Ft. Collins, CO 80523-1676 Attn: Joni Phone: 970/491-2379 Fax: 970/491-2940	LAB USE ONLY JTPID:_____ Date Rc'd:_____ Location:_____
Veterinarian or contact person:	Animal owner:
Clinic/Institution:	Number of samples submitted:
Address: e-mail:	Phone: Fax:
City:	State: / Zip code:

Animal ID	Sex	Age	Species	TB Skin Test Results	Other TB Test Results	Date Sample Taken

Sample Submission:

1. Samples must be collected by a licensed veterinarian.
2. Samples must be submitted in 12 mm x 75 mm serology tubes, or 1.7 ml microcentrifuge tubes.
3. Shipping boxes must say "Refrigerate Upon Arrival."
4. Collect blood into red top clotting tube, allow to clot, centrifuge, transfer serum into another vial, and send at 4 C or frozen.

Appendix 2. VS Form 10-4 (front)

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONAL VETERINARY SERVICES LABORATORIES
AMES, IOWA 50010

SPECIMEN SUBMISSION

INSTRUCTIONS: Use a separate request for each species and each owner/broker. See reverse of Part 5 for definitions *(item 11)* and instructions for identification *(item 19)*.

PAGE ____ OF ____

1. NAME OF SUBMITTER *(Last, First, MI)*

MAILING ADDRESS *(Street, City, State, Zip code, and telephone no.)*

2. NAME OF OWNER/BROKER *(Last, First, MI)*

CITY | STATE

3. LOCATION OF ANIMALS

County | State

4. HERD/FLOCK SIZE

5. NO. IN HERD/FLOCK AFFECTED

6. NO. IN HERD/FLOCK DEAD

7. EXAMINATIONS REQUESTED *(Diseases and Test Procedures)*

8. COLLECTED BY

9. DATE COLLECTED

10. AUTHORIZED BY

11. PURPOSE OF SUBMISSION *("X" one) (See reverse side of Part 5 for definitions)*

☐ General Diagnostic ☐ Surveillance/Monitor ☐ Import
☐ FAD/EP Diagnostic ☐ Developmental/Research ☐ Export
☐ NVSL Intralab Diagnostic ☐ Reagent Evaluation

12. COUNTRY OF ORIGIN/DESTINATION

13. REFERRAL NUMBER

14. PRESERVATION *("X" applicable item(s))*

☐ None ☐ Ice Pack ☐ Dry Ice ☐ Formalin ☐ Borax ☐ Alcohol ☐ Other *(specify)*

15. SPECIMENS SUBMITTED *("X" applicable item(s))*

☐ Blood ☐ Feces ☐ Parasite ☐ Serum ☐ Tissue ☐ Whole Bird
☐ Culture ☐ Feed ☐ Plant ☐ Soil ☐ Urine ☐ Other *(specify)*
☐ Extract ☐ Milk ☐ Semen ☐ Swab ☐ Water

16. TOTAL NUMBER OF SPECIMENS SUBMITTED

17. SPECIES OR SOURCE *("X" one)*

☐ Cattle ☐ Goat ☐ Environment ☐ Chicken ☐ Other *(specify)*
☐ Swine ☐ Horse ☐ Reagent ☐ Turkey
☐ Sheep ☐ Donkey ☐ Pet Bird *(specify)*

18. NUMBER OF ANIMALS SAMPLED

19. IDENTIFICATION *(see reverse side of Part 5)*

Sample ID	Animal ID	AGE	SEX	Sample ID	Animal ID	AGE	SEX

20. ADDITIONAL DATA *(History, clinical signs, post mortem findings, remarks, tentative diagnosis, etc. Use additional sheets if necessary. Attach 5 copies.)*

21. SIGNATURE OF SUBMITTER AND DATE SUBMITTED

NVSL USE ONLY

CONDITION	PRIORITY	DISTRIBUTION	RECEIVED BY

NVSL ACCESSION NO.

VS FORM 10-4 (MAY 93) *Previous edition obsolete.*

PART 5 - SUBMITTER

Appendix 2b. VS Form 10-4 (back)

Item 11 - Definitions of Diagnostic Case Categories

General Diagnostic Case - A case in which the tests conducted are for the purpose of diagnosing or confirming a domestic disease, and/or the analysis of environmental products that may be contributing to an existing disease condition.

FAD/EP Diagnostic Case - A case in which the tests conducted are for the differential diagnosis or confirming a foreign disease, or for the eradication of a foreign disease that has gained entrance into the U. S.

NVSL Intralab Diagnostic Case - A case in which the tests conducted are for the purpose of diagnosing or confirming a disease condition, analyzing environmental products that may be contributing to a disease condition or for analyzing chemical products for another laboratory of NVSL.

Surveillance/Monitor Case - A case in which the tests conducted are for the purpose of monitoring for a specific disease, for a specific insect or insect vector, or for analyzing specific products that are used in treating animals or poultry or for decontamination of animal or poultry facilities.

Developmental/Research Case - A case in which the tests are conducted for the purpose of supporting a developmental or research project conducted by another laboratory of NVSL, by staff or field personnel of VS or by other laboratories, institutions, or agencies.

Reagent Evaluation Case - A case in which the tests conducted are for the purpose of evaluating a reagent produced by another laboratory of NVSL or by other laboratories, institutions, or agencies.

Import Case - A case in which the tests conducted are for the purpose of qualifying animals or poultry, including wild animals and birds, or animal or poultry products for importation into the U. S.

Export Case - A case in which the tests conducted are for the purpose of qualifying animals or poultry, including wild animals and birds, or animal and poultry products for exportation to a foreign country.

Item 19 - Identification

Identify Samples with Consecutive Numbers - Record animal identification *(number or name)* adjacent to appropriate sample number. Laboratory results will be reported by sample identification number. Indicate approximate age in years(y), months(m), weeks(w), or days(d), and indicate sex of each animal. See example below. When more than 10 samples, use VS Form 10-4A.

IDENTIFICATION		AGE	SEX	IDENTIFICATION		AGE	SEX
Sample	Animal			Sample	Animal		
1	12ABC0000	3y	F	6	12ABC0005	10d	F
2	12ABC0001	2y	M	7	12ABC0006	10m	F
3	12ABC0002	1y	F	8	12ABC0007	8m	M
4	12ABC0004	6m	F	9	12ABC0008	2½y	F
5	12ABC0005	3w	M	10	12ABC0009	15m	M

Appendix 3. Ungulate Necropsy Protocol (General)

Institution/Owner_____

Address _____

Address _____ Country _____

Species _____ ID# _____ ISIS# _____ Studbook#_____

Birth date/Age _____ Sex _____ Weight (kg)_____(actual/estimate)

Death date _____ Death location _____

Necropsy date _____ Necropsy location _____ PM interval _____

Captive-born? _____ Wild-caught? _____

History (Include clinical signs, circumstances of death, clinical labwork, diet and housing)

GROSS EXAMINATION
(If no abnormalities are noted, mark "normal" or "not examined" (NE)

General Exam (Physical and nutritional condition, pelage, SQ fat, body orifices, superficial lymph nodes)

Musculoskeletal system (Bones, marrow, joints, muscle)

Body cavities (Fat stores, pleura, thymus, lymph nodes)

Spleen

Respiratory system (Nasal passages, pharynx, larynx, trachea, bronchi, lungs, regional lymph nodes)

Appendix 3 (cont.). Ungulate Necropsy Protocol (General)

Cardiovascular system (heart, pericardial sac, great vessels, myocardium, valves, chambers)

Digestive system (mouth, teeth, tongue, esophagus, stomach, small and large intestine, anus, liver and gallbladder, pancreas, mesenteric lymph nodes)

Urinary system (kidneys, ureters, bladder, urethra)

Reproductive system (testes/ovaries, uterus and cervix, penis/vagina, accessory sex organs, mammary gland, placenta)

Endocrine system (thyroids, parathyroids, adrenals, pituitary)

Central nervous system (brain, meninges, spinal cord)

Sensory organs (eyes, ears)

Additional comments or observations:

Prosector _____ Date: _____

Summarize preliminary diagnoses:

Laboratory studies (Results of cytology, fluid analysis, urinalysis, serum chemistries, bacteriology, mycology, virology, parasitology, x-ray, photography, other)

Appendix 4. Tissue Checklist

Where possible freeze 3–5 cm blocks of tissue from major organs (e.g., lung, liver, kidney, spleen) in small plastic bags, preferably in liquid nitrogen to be kept ultrafrozen at –70 degrees Celsius; freezing at conventional temperatures is acceptable if there is no access to an ultrafreezer. See Appendix 5 for special handling of ungulate TB suspects.

Preserve as many of the following tissues as possible in 10 percent buffered formalin at a ratio of approximately 1 part tissue to 10 parts solution. Tissues should be no thicker than .5 to 1 cm. Also, fix diced 1×1 mm pieces of kidney, liver, spleen and lung in a suitable EM fixative, if possible, gluteraldehyde base e.g., Trump-McDowell fixative. NOTE: There is generally no need to fix and label each tissue separately. Take two sets of fixed tissue, one for the primary pathologist, and the other for the regional/SSP pathologist. Send tissues required for diagnosis to primary pathologist and request a duplicate set of slides for the regional/SSP pathologist who should be contacted for further instructions. Also, freeze post-mortem serum (from heart), urine, and any abnormal fluid accumulations. Consult Special Projects Protocol for any special instructions about specimens requested by the designated researcher.

- ❑ Brain
- ❑ Nerve (Sciatic)
- ❑ Spinal Cord
- ❑ Eye
- ❑ Tongue
- ❑ Trachea
- ❑ Thyroid
- ❑ Parathyroid
- ❑ Pituitary
- ❑ Joint capsule
- ❑ Heart
- ❑ Muscle
- ❑ Lung
- ❑ Diaphragm
- ❑ Liver
- ❑ Gall Bladder
- ❑ Spleen
- ❑ Pancreas
- ❑ Small Intestine
- ❑ Large Intestine
- ❑ Cecum
- ❑ Skin
- ❑ Adipose tissue
- ❑ Aorta
- ❑ Bone with marrow
- ❑ Bulbourethral gland
- ❑ Testis/Ovary
- ❑ Uterus
- ❑ Mammary gland
- ❑ Ureter
- ❑ Urethra
- ❑ Kidney
- ❑ Adrenal
- ❑ Thymus
- ❑ Prostate
- ❑ Seminal vesicles
- ❑ Lymph nodes
- ❑ Salivary gland
- ❑ Scent gland
- ❑ Epididymis

Primary pathologist: Name _____

Lab _____

Address _____

_____

Phone ( ) _____

(Please attach final pathology report and send a copy with this protocol to the regional/SSP pathologist.)

Appendix 5. Ungulate Necropsy Protocol (TB Suspects)

A complete systematic necropsy should be performed according to the Ungulate Necropsy Protocol (App. 3), taking into consideration the following steps for potentially tuberculous animals.

An intense search for TB lesions is encouraged in the following categories:

1. Individuals that die or are euthanized and are proven or suspected of having TB antemortem; and
2. Animals that die or are euthanized from herds with TB suspect(s) or that have had contact with tuberculous animals.

Since *M. tuberculosis* complex organisms (including *M. bovis* and *M. tb*) are potentially infectious to humans, proper protective apparel must be worn by the prosector, and any suspicious organs or lesions should be contained as soon as possible.

- Ideally, ungulates for elected euthanasia should be tuberculin skin-tested and bled for ELISA and tracheal washes or bronchoscopic samples taken just prior to euthanizing. For elephants in any of these categories see special SSP Elephant Necropsy Protocol and USDA Guidelines for the Control of Tuberculosis in Elephants.
- Ungulates undergoing necropsies should have a careful examination of the tonsillar regions and submandibular lymph nodes for tuberculous appearing lesions. Take any lymph nodes that appear caseous or granulomatous for acid-fast staining, DNA amplification testing and TB culture, and freeze or ultra-freeze one-half in plastic whirl-packs. (Do not use borate solution sometimes used for TB submissions in cattle). Label and preserve the other half in buffered 10 percent formalin for histopathology.
- If there are no TB type lesions readily apparent, thoracic organs should undergo a very careful search for early stages of TB as follows: When the lungs and trachea are removed, find the bronchial lymph nodes at the junction of the bronchi from the trachea. Use clean or sterile instruments and slice the lymph nodes and take half for TB culture (even if no lesions are evident), and sections for fixation.
- Carefully palpate the lobes of both lungs from the apices to the caudal borders and slice into areas where firm nodular lesions might be felt. Take sections of any lesions for culture, DNA amplification and fixation as noted above. Open all bronchi well into the parenchyma, then bread-loaf all lobes and similarly take any suspicious lesions. Open the trachea and look for nodules or plaques and process as above. Regional thoracic and tracheal lymph nodes should also be examined and processed accordingly.
- Look for and collect possible extra-thoracic TB lesions in other organ systems particularly if there is evidence of advanced pulmonary TB and process as above.
- Submit the complete set of fixed tissues to your primary pathologist. For further information about processing of samples for TB diagnostics and cultures and where to send them please consult the guidelines in Chapter 5 (this volume).
- Report all ungulate necropsies positive for TB to the appropriate SSP or TAG veterinary advisors and to the NTBWG as soon as possible using the form in App. 7 (this volume).

Appendix 6. Diagnostic Test Contact Information

Dr. Richard Montali
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Department of Pathology
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Washington, DC 20008-2598
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F: 202-673-4660
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Appendix 7. Paper TB Test Reporting Form