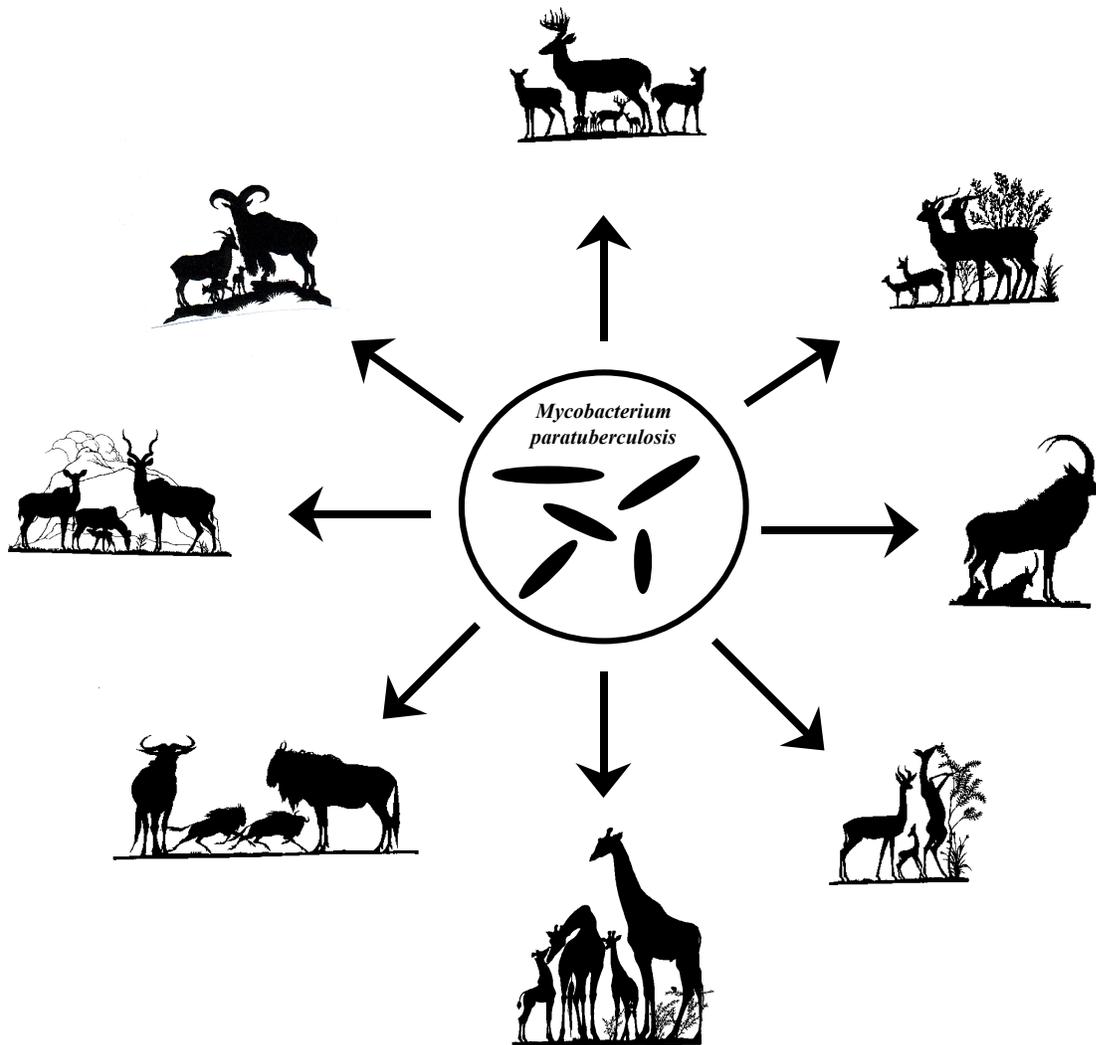


# Proceedings of the Workshop on Diagnosis, Prevention, and Control of Johne's Disease in Non-Domestic Hoofstock

White Oak Conservation Center  
Yulee, Florida  
June 26-28, 1998



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### INTRODUCTION

Johne's disease (JD) is an insidious wasting illness of Artiodactylids. Also called paratuberculosis, this chronic wasting disease is caused by infection with the organism, *Mycobacterium paratuberculosis* (Mptb). JD was first described in 1895 and has spread in domestic livestock, proving especially costly for the dairy industry. Data from the 1996 National Animal Health Monitoring System survey indicated that approximately 40% of dairy cow herds with greater than 300 animals were infected with JD.

Zoos accredited by the American Zoo and Aquarium Association (AZA) have long maintained programs of veterinary health care and disease surveillance as part of their commitment to the well-being of the rare animals in their collections. Members of AZA accredited institutions have looked on with some concern at the alarming dissemination of JD infection that has been experienced in the domestic livestock industry. Zoo veterinarians, who attended this workshop as members of the American Association of Zoo Veterinarians (AAZV), believe that adoption of JD prevention and control protocols will help insure the continued health of Artiodactylid collections in zoos and other captive settings.

On June 26-28, 1998 a group of zoo veterinarians, pathologists and zoo animal managers met with experts in JD diagnosis and control as well as representatives from the North American wildlife disease community. This group gathered in a consensus building workshop to establish voluntary guidelines for veterinarians and animal managers in the zoo setting. Though not presently believed to be a major problem in the majority of zoological collections, it is hoped that this document will increase the zoological community's awareness of the potential threat this disease poses to its collections and to the benefits of JD surveillance programs. Once this often *invisible and insidious* disease is established, it can be extremely difficult to control. We believe active surveillance, prevention, and control is needed to maintain the health of rare zoo animals, limit the costs (time, money and labor) of infection, and prevent

the loss of valuable genetic representatives. The adoption of these guidelines will generate the data needed to ascertain the present prevalence of the disease in zoological collections, prevent the establishment of JD as a disease of significant concern in zoological collections, and control and eradicate the disease where it might be found in zoological collections. As with any health program, prevention pays: it is more effective and less costly to safeguard animals from infection than it is to manage animals with the disease.

## OVERVIEW AND GENERAL CONSIDERATIONS

The information, suggestions, and guidelines presented in this proceedings are the results of discussions and consensus building among the group of participants at this workshop and are a reflection of the opinions of this group at the time of the workshop was held. These opinions are subject to different interpretations so we recommend that, before proceeding with any plans based on the enclosed information, you validate your interpretation with the most current information available.

All zoological institutions need to be aware of and be wary of JD. The zoological community as a whole would benefit from knowing the current prevalence of the disease in the national collection. With this in mind, and knowing the enormous potential costs of an undetected spread of Mptb infection, the working group has outlined voluntary methods by which to:

- assess the current prevalence of this disease in captive collections.
- determine to which of three categories the collection animals belong (low risk, high risk or unknown JD status).
- establish a control or monitoring program according to risk category.
- highlight the risks of JD transmission when considering animal transfers.
- educate all veterinary and animal handling personnel about JD.
- support research efforts to improve JD control in captive and wild Artiodactyla.

### A. Definitions

1. **Johne's Management Unit ("JMU"):** For purposes of diagnostic testing, surveillance, control and animal movement in Artiodactylids susceptible to Mptb, the JMU is defined as the animal, species, enclosure, geographic area, or institution of concern. Each facility must identify and define JMUs within the facility's management scheme.
2. **Culture Test (+) :** Isolation and identification of Mptb from feces or tissue using either conventional or radiometric mycobacteria culture methods plus DNA probe for IS900 to confirm the identity of the isolate as Mptb. A culture test (+) tissue sample along with pathology consistent with JD would confirm a Mptb infection in virtually all cases. A culture test (+) fecal sample does not necessarily confirm a Mptb infection. Possible explanations for a fecal culture test (+) result include:

- a. The animal ***is infected*** and shedding the organism. The animal can display:
    - i. Clinical Signs: detectable lesions/acid-fast organisms in tissue **or**
    - ii. No clinical signs (pre-clinical or subclinical infection) with/without detectable lesions/acid fast organisms in tissue.
  - b. The animal ***is not infected***: Mptb organisms from contaminated feed, water, or soil are “passing through” the uninfected animal.
3. **Mptb Infection:** Culture test (+) plus histologic lesions compatible with Johne’s Disease. The animal may or may not have clinical signs compatible with Johne’s disease.
  4. **Culture Test (-):** No isolation of Mptb from feces or tissue using either conventional or radiometric mycobacteria culture plus DNA probe for IS900 to confirm the identity of the isolate as Mptb. *Does not rule out the possibility of M. paratuberculosis infection within the JMU.* Possible explanations for a culture test (-) result are:
    - a. Uninfected animal.
    - b. Infected animal not shedding organisms (i.e., early infection).
    - c. Infected animal shedding organisms at times, but not detected by culture due to intermittent shedding, sampling error (happened to pick up an organism - free sample), laboratory error, or tested by an insensitive culture technique.
    - d. Previously infected animal now “recovered” and not shedding organisms (considered a very rare event).
    - e. Mptb strain with characteristics such that traditional culture media does not support its growth (i.e, sheep strain of Mptb is distinct and does not grow well on traditional medias).
  5. **Criteria for Diagnosis of JD in an Individual Animal**  
Tissue culture (+) OR multiple (>2) fecal culture (+) OR single fecal culture (+) AND at least one of the following:
    - a. Clinical signs of wasting compatible with JD,
    - b. Histologic findings (lesions/acid-fast organisms) compatible with JD, **or**
    - c. JMU history of infection within the last year.
  6. **Criteria for Suspicion of JD in an Individual Animal**  
Any **one** of the following:
    - a. Compatible clinical signs with single fecal culture (+),
    - b. Histologic findings compatible with JD,
    - c. JD infection confirmed in dam or offspring, **or**
    - d. Co-habitation with known infected animals.
  7. **Histologic Lesions Compatible with JD:** Any histiocytic or granulomatous inflammation in the gastrointestinal tract or mesenteric lymph node(s) which has acid-fast bacilli present. While such lesions are not pathognomonic for JD, they may be considered sufficient for confirmation of a clinical case.

8. **Histologic Lesions Suspicious for JD:** Any epithelioid macrophages or multinucleated giant cells in the lamina propria of the intestine (any level), or mesenteric lymph node(s), which are negative for acid-fast bacilli AND which lack any intracytoplasmic crystalline material, pigment, or debris. Such suspicious cases could be resolved (confirmed positive or negative) by additional tissue testing (e.g., immunoperoxidase stains or PCR) along with further interpretation of histopathology in light of additional background, epidemiologic data, or heightened surveillance in the herd.
9. **Clinical Signs Compatible with JD:** The presentation of JD in non-domestic Artiodactylids can be markedly different from that of domestic livestock. Clinical signs can range from an inapparent Mptb infection to the more typical domestic livestock presentation of weight loss, poor hair coat, and chronic diarrhea. It is therefore most important to perform appropriate testing prior to making a diagnosis of JD in non-domestic Artiodactylids.

## B. Education of Institution's Staff

Staff education is the cornerstone of any good disease surveillance effort. The initiation of a JD prevention and control program should begin with education and effective communication. The veterinarian responsible for the health of the collection must learn as much as possible about the disease. The essence of these investigations should then be passed on to the facility's staff via lectures, discussions, and written materials:

1. **Husbandry and Management Staff:** Explain the nature of the disease, the potential risks to the institution, importance of reducing the risks of disease spread through improved husbandry and sanitation, the insidious nature of the disease, the inherent difficulties in test interpretation, potential impact of the disease on the health of the collection and management capabilities, and the benefits of a thorough surveillance program. Stress the need to define risks within the institution and develop preventive measures to reduce or eliminate these risks. Staff should be kept abreast of current scientific thought on the zoonotic potential of Mptb.
2. **Maintenance Staff:** Should be made aware of how work activities in and around JMUs and movement through JMUs may impact prevention and control programs.
3. **Administrative Staff:** As above, plus analysis of potential impact on future institutional goals (i.e., policy and status with regards to domestic and international animal exchanges and public health and relations) and cost and benefits of instituting a thorough surveillance and/or control program. Demonstrate the financial, managerial and animal health value of instituting preventive measures.
4. **Public Relations Staff:** Outline the impact of control and eradication programs on individual high profile animals or exhibits and on domestic and international marketing programs. Discuss the status of current scientific thought on the zoonotic potential of Mptb.

## C. General Preventive Measures

The following measures help to prevent the introduction or spread of the disease. This is a good starting point, even in collections which have not yet begun surveillance programs and will be beneficial, should the need for control programs be necessary:

1. **Identify all Artiodactylids past weaning age with permanent identification** (i.e., transponders, ear tags, tattoos, ear notches, etc.) in order to establish preventive measures and to start surveillance in animal collections.
2. **Evaluate Known Risk Factors** for Introduction and Dissemination of JD and **Develop Preventive Measures to Reduce or Eliminate Risk**
  - a. **Identify Animal Sources of Risk**
    - **Review Clinical Cases and Demographics of Collection**
      - C What species are involved?
      - C Identify JMUs within facility. What JMUs are involved?
      - C Identify risk groups exposed to known cases.
      - C Review all cases of chronic weight loss and/or diarrhea in the last five years to confirm the diagnoses, recalling that other GI diseases can mask the JD diagnosis.
      - C Review Artiodactylid pathology cases from the last five years for gross and/or histologic lesions compatible with JD.
      - C Assess any undiagnosed cases consistent with JD in more detail to include demographics.
      - C Isolate animals with chronic weight loss and/or diarrhea from herds and evaluate thoroughly for JD.
    - **Review History of JMUs**
      - C How long has the JMU been closed to animal movements?
      - C Have there been animal movements in and out?
      - C Minimize animal movements between JMUs.
      - C Evaluate “locational or extended” risk of exposure between JMUs.
      - C Institute effective preventive medicine programs for JMUs.
    - **Review and Evaluate History of New Arrivals**
      - C When and what species?
      - C Past medical history and origin of new arrival with special emphasis on JD history of the sending facility.
      - C Institute effective quarantine and health evaluation programs for new animals.

– **Evaluate Contact or Association with Wildlife**

- C What wildlife species have access to JMUs?
- C Wild ruminants, swine, and rabbits are known potential Mptb carriers
- C Other feral animals could possibly transfer the organism from one area to another (i.e., birds, rodents, etc.)

– **Review Domestic Livestock Contacts In or Near Institution** (including petting zoo, adjacent domestic livestock facilities or ranched non-domestic livestock).

- C Non-domestic Artiodactylids should be kept isolated from domestic livestock.
- C Water Sheds shared with domestic livestock should be fenced off.
- C Domestic livestock for petting zoos should be obtained whenever possible from JD-free herds. Consider having animals pre-tested prior to purchase.
- C Institute effective preventive medicine and health evaluation programs for domestic livestock in petting zoos.

**b. Identify Management Sources of Risk**

– **Identify Animal Movements** that may increase risk of disease transmission

- C There is less risk trading between known test negative JMUs. Institutions which receive animals from JMUs of unknown status should realize that their own JMU will then revert to unknown status, requiring re-initiation of screening/surveillance.
- C Thorough records should be kept of animal movements into and between JMUs.
- C Minimize animal movements between JMUs, direct contact sites between JMUs, and common holding and shifting sites between JMUs.

– **Examine Management Schemes**

- C There may be more inherent risks in certain management schemes (i.e., mixed species JMUs).

– **Review Sanitation Techniques**

- C Thorough manure removal where possible, particularly in areas of high exposure such as birthing, feeding and watering, and congregation areas.
- C Use of disinfectants effective against Mptb (i.e., phenolics).

– **Review Calf Management Techniques**

- C Feed only “low risk” colostrum (i.e., test negative dairy herds, freeze-dried supplements) or use plasma transfusion to boost passive immunity.

**– Identify Situations Where Feed May Be Contaminated**

- C Do not feed manure-contaminated forage.
- C Feed animals off ground and over cement.
- C Move feeding areas around to reduce contamination.

**c. Identify Environmental Sources of Risk**

**– Soil**

- C Alkaline soils may reduce risk of spread (i.e., lime pastures).
- C Reduce fecal contamination of soil where possible, especially in shaded and/or moist areas.

**– Water**

- C Remove or reduce standing water and wallows in JMUs.
- C Prevent animal contact with bodies of water receiving runoff from other JMUs or agricultural sources.
- C Prevent manure contamination of water sources.

**– Fomites**

**C Equipment**

- < Reduce transfer of cleaning equipment from one JMU to another.
- < Clean soles of boots off between JMUs (i.e., hosing, use of foot baths).

**C Feeders**

- < Reduce transfer of food bowls and utensils between JMUs.
- < Thoroughly clean and disinfect food bowls and utensils between feedings.

**C Vehicles**

- < Reduce vehicular traffic through and between JMUs.
- < Shift travel patterns to avoid “high risk” areas.

**3. Initiate and Maintain Diagnostic Testing**

- a. Mycobacterial culture of feces or tissue (radiometric or conventional) is, at present, the only recommended antemortem test for diagnosis and surveillance of JD in non-domestic hoofstock. Positive culture should always be confirmed by DNA probe (IS900). All Mptb isolates should be saved for future JD research. Although the Agar Gel Immunodiffusion Test (AGID) has low sensitivity, it may be used in clinical cases to support a diagnosis of Mptb infection.
- b. Only culture results from laboratories accredited for JD testing should be accepted. Check that laboratories assure the confidentiality of test results.
- c. For domestic hoofstock maintained in zoological institutions (i.e. petting zoo and farm-in-zoo animals), Enzyme Linked Immunosorbent Assay Test (ELISA) may

be performed on goats and cattle, and AGID for sheep and llamas as a less costly, but potentially less accurate, alternative to culture. Hand-reared non-domestic hoofstock maintained in petting zoos or farm-in-the-zoo facilities should be tested using the radiometric or traditional culture methods.

## **INITIAL SURVEILLANCE PROGRAM FOR LOW RISK OR UNKNOWN STATUS COLLECTIONS OR JMU<sub>s</sub> WITHIN A COLLECTION**

Institutions that have not had clinical cases of JD and which have performed quality necropsy and histopathology survey exams of non-domestic hoofstock for at least five years without seeing evidence of JD infection would be considered **low risk** as they begin a program of surveillance. Institutions which have not maintained good medical records and/or which have not performed routine necropsy and histopathology exams would be considered **unknown status** as they begin a program of surveillance. In either case, this does not diminish the need for a surveillance program.

**The prevalence of JD in a given JMU is the most valuable diagnostic information and is more important than an individual animal test result. JMU surveillance should be made the highest priority for all institutions, whether they have identified JD cases in the past or not.** A single or even multiple negative tests on an individual animal does not assure lack of JD in the individual or the negative status of the JMU. A JD surveillance program should be based on risk assessment, available resources, and appropriate aggressiveness to move steadily toward institutional goals.

- A. Testing to Ascertain JMU Status: A number of schemes are provided as examples of possible surveillance strategies and should be considered in context of prior risk assessment. Each strategy is for an individual JMU within an institution. Each has advantages and disadvantages within certain management operations.
- 1. Perform one JD Fecal Culture on all mature animals, individually identified and performed over the shortest interval possible within a JMU:** The advantages include providing the most thorough picture of potential infection of the JMU as well as being able to individually identify a culture positive animal. This strategy also reduces the length of time a possible shedder will have to contaminate the environment. The disadvantages include the labor involved to obtain samples from individually identified animals and the cost of culturing each individual. Based on risk assessment, the most advantageous timing for sample collection should be chosen. **Statistical evaluation indicates that this is the recommended method to gain the most realistic estimate of prevalence in the JMU.**
  - 2. Perform one JD Fecal Culture on 50% of the mature animals (include any animals with suspect status), individually identified, in each JMU over a 30 day period:** The advantages are decreased labor and costs. The disadvantage is that infected animals are more likely to be missed and will continue to contaminate the environment. If JD prevalence is low, this method reduces the opportunity for control at the earliest, most effective, and cheapest point in time. **This method will not provide as complete a picture of the JMU<sub>s</sub> status as option #1.**

3. **Perform JD Fecal Cultures on random stools (not identifying individuals) totaling 50% of the number of mature animals in the JMU at one point in time:** The advantages are a significant decrease in labor as compared to the collection of individually ID samples and a decrease in costs per year. The marked disadvantage is that should there be a positive culture, it will not be possible to identify the individual. Should the unknown individual be an intermittent shedder of Mptb, it may never be identified, and the JMUs status will remain in question. **This method is the least reliable and should only be considered as a first step in a long range plan to evaluate JMU prevalence.**
- B. **All post-weaning, JD susceptible, animals that die and whose carcasses are recoverable in adequate post-mortem condition should be necropsied.** Histopathologic evaluation of representative sections of the duodenum, jejunum, ileum (including Peyer's patches, if present), cecum, colon and mesenteric lymph nodes (including ileocecal lymph node) is recommended. In addition it is recommended that at necropsy, **three mesenteric lymph nodes, one of which is the ileocecal lymph node should be submitted for mycobacterial culture.** If the cost of three lymph node cultures is prohibitive, the three lymph nodes may be pooled for a single culture rather than submitting a single lymph node for culture.
  - C. **Following completion of one of the above surveillance programs (A1 to A3) and, if there are no positive fecal cultures and no animals are diagnosed with JD, then the JMU is considered TEST NEGATIVE. GO TO MONITORING PROGRAM FOR JMUs WITH A TEST NEGATIVE STATUS (Pp.47-48).**
  - D. **If one of the above surveillance programs (A1 to A3) is completed and no positive fecal cultures are found, but there are suspicious histopathology lesions (this would include acid-fast bacilli or multinucleated giant cells or epithelioid macrophages in lymph node(s), but acid-fast negative) then further confirmatory testing should be performed on the tissues (immunostains, PCR, or reinterpretation in light of additional background and epidemiological data) before deciding on the JMUs status.**
  - E. **If positive culture results are obtained from a JMU or an animal in the JMU has been diagnosed with JD, then go to CONTROL PROGRAMS FOR INFECTED JMUs.**

### **CONTROL PROGRAMS FOR INFECTED JMUs**

Control programs should be initiated for JMUs which are deemed infected. The determination of an infected JMU is based on the evaluation of culture results, JMU health history, post-mortem examination, and histopathology results.

- A. **Assemble a team to develop and implement a control plan.** Experts in the field (Diagnosticians, Pathologists, Epidemiologists, etc.), Veterinary Staff, Upper Management, Curatorial Staff, Keeper Staff and Exhibit Maintenance Staff.
  1. **Review and, if necessary, revise the JD education program.**
- B. **Identify risk factors and implement plans to minimize risk of transmitting JD outside the JMUs (Review General Preventive Measures, Pp.40-43):**

- **Sanitation** - intensify removal of feces (fecal pick-up crews, turf sweepers), use of phenolic disinfectants, separate or disinfect cleaning utensils, and boot or shoe cleaning and disinfection.
- **Discontinue animal movement** into and from the JMU until it is established as a test negative maintenance surveillance JMU.
- **Limit traffic** flow through JMUs.
- **Feeding strategies** - Clean and disinfect feeders and waterers, feed off of ground and over cement, check forages for fecal contamination, and move feeding sites regularly to reduce contamination.
- **Calf management** - separation and hand-rearing of calves, colostrum management (from certified domestic herds, freeze-dried supplements).
- **Environmental management** - limit exposure to contaminated water and wallows, improve and re-route drainage of JMUs, prevent exposure to agricultural run-off, and alkalize soils (lime).
- **Modify breeding plan** in the JMU - stop or limit breeding.

**C. Develop record and management system to store, track and retrieve data:**

- MedArks
- Other

**D. Notify tracebacks 1-2 years.**

**E. Develop Testing Strategy to Determine Prevalence within and between JMUs:**

To assist in focusing resources, it is important to determine the infection prevalence in the collection. This includes finding the number of test-positive/clinical animals, number of exposed animals including offspring from test-positive/clinical animals plus routes of likely transmission.

1. **Test individuals of an infected JMU:** The more aggressive the testing program the higher the confidence in the results and the more likely one will reduce the risk of spread within the JMU.
  - a. The minimum recommended testing regimen would include one Mptb fecal culture per animal per year. All samples must be collected from individually-identified animals.
  - b. Optimally, testing would include two or more cultures per individually-identified animal per year.
  - c. Testing should be done at the most advantageous time to allow for effective decision making.

**F. Actions based on Test Results:**

1. Animals which are test positive (including offspring of test positive dams and dams of test positive offspring) should be removed from the JMU.

2. If a large percentage of a definable group of animals are test positive, it may be advisable to remove that entire group from the JMU. For example: If a single round of JD culture results reveal 30% or higher test positive individuals, all the animals within the JMU should be removed.
3. The removal of test positive individuals may be accomplished by:
  - a. Euthanasia or group depopulation
  - b. Long-term isolation - to salvage genetic material or for JD research purposes.
  - c. Isolation and treatment - Should be considered only for animals showing clinical signs in order to salvage genetic material or as a research tool.

**G. The goal of a Control Program is for all JMUs in the institution to be test negative. Once this goal has been achieved, a MAINTENANCE SURVEILLANCE PROGRAM based upon the control team's assessment should be initiated. The following are key aspects of a maintenance surveillance program:**

1. Monitoring Incoming and Outgoing Animals (See Animal Transfer Guidelines).
2. Continued Monitoring of Collection through annual testing regimen.
  - a. Testing scheme and frequency of testing based upon control team's assessment.
3. Continued Attention to Minimizing Risk Factors and Implementing Education Programs.
4. Monitor and Modify Plan as needed and as new information becomes available.
5. Team Decision to Proceed to Monitoring Program (See Monitoring Program for JMUs with a Test Negative Status below).

#### **MONITORING PROGRAM FOR JMUs WITH A TEST NEGATIVE STATUS**

- A. Artiodactylids past weaning age must have permanent identification.**
- B. New animals placed in these JMUs must be from another JMU with the same level of surveillance and with the same test result status.**
- C. Animals with signs consistent with JD (i.e., such as those seen in domestic livestock of weight loss, poor hair coat, and chronic diarrhea) must receive a veterinary medical assessment which may include a specific evaluation for JD.**
- D. All post-weaning animals that die and whose carcasses are recoverable in adequate postmortem condition should be necropsied.** Histopathologic evaluation of representative sections of the duodenum, jejunum, ileum (including Peyer's patches, if present), cecum, colon and mesenteric lymph nodes (including the ileocecal lymph node) is recommended in addition to culture.

- E. **On necropsy, three mesenteric lymph nodes, one of which is the ileocecal lymph node, should be submitted for mycobacterial culture.** If the cost of three lymph node cultures is prohibitive, the three lymph nodes may be pooled for a single culture which is preferable to submitting a single lymph node for culture.
  
- F. **In general, JMUs with the highest number of risk factors should be targeted for increased surveillance (greater testing frequency)**
  - 1. JMUs with more animal transfers
  - 2. Mixed species JMUs
  - 3. JMUs with exposure to domestic ruminants or agricultural run-off
  
- G. **Perform JD fecal cultures on a percentage of post-weaning animals in each JMU on a rotating basis.** The percentage of animals tested annually in each JMU should be based on a thorough risk assessment.

### **ANIMAL TRANSFER GUIDELINES**

The decision to transfer animals is made between institutions. The receiving institution must decide whether the risk of exposure to JD is offset by the importance of the animal transfer. A risk assessment is performed by the receiving institution's management staff and veterinarian in consultation with the shipping institution's counterparts. To follow are suggestions for what information is necessary to make an informed decision:

- A. **Minimum requirements of the shipping institution:** These are tasks to be required of the shipping institution by the receiving institution:
  - 1. Perform general health assessment of animal(s) and comply with state and federal regulations. The animal(s) should be accompanied by a health certificate signed by the veterinarian of record for the shipping institution.
  - 2. Provide medical records and evidence of permanent identification.
  - 3. Describe JD Surveillance program and any JD history in the source JMUs. Test results from all the animals in the source JMUs are more important than negative test results of the individual animal to be shipped.
  - 4. The shipper should arrange direct transport of animal(s) (i.e., NO off-loading en route) using clean, uncontaminated shipping compartments.
  
- B. **Additional recommended requirements of the shipping Institution:** The receiving institution may want to consider requiring these additional criteria from the shipping institution:
  - 1. Preshipment physical examination including baseline laboratory work (hematology and

serum chemistry evaluation).

2. Medical records sent in advance of the animal.
3. Written Program for JD Surveillance.
4. That these animals be transported with animals of similar JD status (request of transporter).

C. **Minimum requirements of the receiving institution:** To minimize the risk of bringing a previously undiagnosed Mptb infected animal from a JD negative facility into the collection of the receiving institution, the receiving institution should:

1. Quarantine or isolate new arrivals for a minimum of 30 days for observation and sample acquisition.
2. Verify that identification of animal matches previous records.
3. Institute individual JD testing, if incoming animals are not from a test negative JMU and minimum requirements of shipping institution were not met. Extend quarantine until test results have been received and are negative.
4. Traceback of animals with positive tests during quarantine and notification of source institution.

D. **Additional recommended requirements for the receiving institution:**

1. Additional individual JD testing regardless of history.
2. Physical examination and routine blood work during quarantine period.

## RESEARCH TOPICS OF VALUE TO ZOOLOGICAL INSTITUTIONS

The working group identified areas of JD research that would benefit the zoological community. Some suggestions took the form of low cost, continuing ways for individual institutions to support research; other suggestions identified topics of particular value for zoos. No attempt was made to identify sources of funding, to detail research design, or to name groups/institutions to perform research.

There is a wealth of information concerning JD diagnosis, epidemiology, pathobiology, etc. in non-domestic species. Unfortunately, the information cannot be capitalized upon in its present form of separate case records from numerous institutions. The historical information and the prospective surveillance data could be compiled on a confidential basis by an AAZV or AZA approved research effort to:

A. **Determine benchmark prevalence in selected species across US Zoos to focus zoological community resources and permit assessment of control efforts through:**

1. Prospective fecal culture surveillance

2. Retrospective assessment and confirmation of clinical cases consistent with JD.
3. Centralized database development and data collection on a confidential basis.
4. Use AZA TAGs and SSPs to perform confidential retrospective data collection including:
  - species test results
  - radiometric and conventional culture result
  - serology results
  - histopathology results
  - clinical case assessment and compatibility with signs
  - tabulate species and methods of diagnosis

**B. Benefit from wild Artiodactyla surveillance efforts:**

Duplication of efforts can be minimized and useful data obtained by cooperating with wildlife specialists who manage their own Artiodactyla surveillance efforts. Suggestions include:

1. Focused sample collection (serum, tissue, feces) to:
  - validate current and new JD serological assays and other methodologies.
  - collect different strains of Mptb for pathogenicity studies.
2. Analysis of necropsy reports to better understand species-specific pathology and disease epidemiology.

**C. Diagnostics**

Improvements in diagnostic assays may provide for better detection of infected animals. The possibilities include:

1. Earlier detection of infected animals via:
  - a. antigen 85 assay
  - b. cellular immunity assays:
    - gamma interferon
    - Intradermal Skin Testing
  - c. molecular genetic assays (nucleic acid probes, PCR)
2. Improved culture methodologies.
3. Validation of diagnostic tests in populations of known status.
4. Development of species-specific diagnostic approaches.

**D. Pathogenesis of Mptb in Multiple Species**

This extensive project would illuminate many aspects of JD control, diagnosis, pathogenicity, epidemiology and the impact of Mptb strain variations. Some of the questions the project would address include:

1. Whether germ plasm from infected animals is infectious.
2. The dose/frequency/delivery protocol necessary to infect various species. This is particularly relevant to environmental control strategies and necessary to assess vaccines.
3. The pattern of immunological responses to infection and fecal shedding of Mptb as displayed by diagnostic test results over time.
4. Species susceptibility to Mptb infection.
5. Pathogenicity of different strains of Mptb.
6. Characteristics during the course of the disease (in-utero infection, clinical signs,etc.)

#### **E. Environmental Controls**

The working group supported research into better methods of managing infective organisms in the environment, to include:

1. Water disinfection and/or filtration trials
2. Fomite disinfection trials
3. Soil management to reduce spread of Mptb
4. Manure (feces) removal and composting

#### **F. Other Interests:**

The working group identified additional areas of research worthy of support including:

1. Improved mycobacterial culture methods
2. DNA fingerprinting of different Mptb strains
3. Vaccination products and strategies
4. Therapeutic protocols; new antibiotic compound testing
5. Zoonotic potential

#### **G. Funding Potential**

Larger institutions should recognize that it may be in their long term best interest to assist smaller institutions, with more limited funding, to test their collections for prevalence of JD. This will allow for continued movement of genetically valuable animals while diminishing the potential for the introduction of JD.

1. **Potential funding sources include:**
  - USDA
  - CEF of AZA
  - Morris Animal Foundation
  - Pharmaceutical Corps
  - EPA - Water testing
  - Individual zoological institution research funding programs

# Johne's Disease Program Flowchart

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