

# Guidelines for Surveillance of Bovine Tuberculosis in Wildlife



**June 2011**



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## **Preface**

The following recommendations were developed to provide guidance for State and Federal wildlife agencies that plan to conduct surveillance for bovine tuberculosis (TB) in wildlife—specifically in areas where new detections of TB are found in dairy, beef cattle, or captive cervid herds.

*These recommendations are meant to be used as a resource during the development of a TB wildlife disease surveillance strategy for sampling wildlife. They are not intended for use in determining the prevalence of disease in wildlife populations. Additionally, the procedures and other information provided in this manual are not mandatory. Farm-specific practices to minimize livestock-wildlife interactions are effective methods of preventing infection; however, those methods are not addressed in this manual. Instead, the guidelines focus specifically on wildlife surveillance strategies for detecting bovine TB.*

*Guidelines for Surveillance of Bovine Tuberculosis in Wildlife* was developed by the Animal and Plant Health Inspection Service (APHIS), in collaboration with wildlife professionals with expertise in bovine TB. These recommendations incorporate the most recent scientific literature and are intended to serve as a starting point for developing and conducting surveillance for bovine TB in geographic areas where the presence of disease in wildlife is unknown. Interagency cooperation is strongly encouraged between State wildlife agencies, other State cooperators, stakeholders, and APHIS' Wildlife Services (WS) and Veterinary Services (VS) to develop an effective area-specific surveillance plan. Additionally, coordination with the National Veterinary Services Laboratories (NVSL) in Ames, Iowa, prior to sampling is also recommended to ensure timely testing of samples.

## **I. Introduction**

### **A. Purpose**

This manual includes recommendations for conducting TB surveillance in wildlife in areas where bovine TB has been detected in a dairy, beef, or captive cervid herds. The main objective of the surveillance strategies described is to determine whether bovine TB is present in wildlife or on surrounding infected premises.

### **TB Detection**

Following the detection of bovine TB in domestic livestock, it is often important to determine if free-ranging wildlife also are infected. If bovine TB is detected in wildlife, an evaluation of risk to domestic livestock associated with the findings of bovine TB in wildlife will be conducted. Depending on the results of the evaluation, a TB management plan may be developed through joint efforts among the State wildlife agency, WS, and VS. These TB management plans should include provisions to further investigate the presence of bovine TB, minimize the spread geographically, increase surveillance, and eventually, eliminate the disease from free-ranging wildlife and domestic livestock.

## Manual Contents

This document includes the following guidelines for obtaining and submitting samples from wildlife:

- Sampling supplies
- Protocols for obtaining samples
- Species to sample
- Sample sizes
- Safety and personal protective equipment (PPE)
- Protocols for shipping samples
- Data management

Questions regarding this manual may be directed to the appropriate contacts listed in Appendix A.

## B. Bovine TB

Bovine TB is a chronic bacterial disease (primarily of cattle) caused by the microorganism *Mycobacterium bovis*. The disease can affect other species, including humans and wildlife. Bovine TB is most often transmitted to humans by inhalation of aerosolized respiratory tract bacteria, ingestion of unpasteurized milk, and inoculation by contaminated instruments (such as knives). The disease can be spread from livestock to wildlife or wildlife to livestock via the fecal-oral route, ingestion of contaminated food, or through the respiratory tract. The APHIS Bovine TB Eradication Program has reduced TB in U.S. cattle; however, spillover into wildlife may maintain the microorganism in the environment and function as a source of re-infection for livestock.

# II. Surveillance Strategies and Sample Collection

## A. Strategies for Identifying Animals for Sampling

### Target Species

The first documented cases of bovine TB in North American wildlife were identified in two white-tailed deer in 1933, one white-tailed deer in 1937, and one white-tailed deer in 1961. All of those cases were from the same area of New York. TB was subsequently found in free-ranging white-tailed deer in northern Michigan in 1975. A second occurrence from the same area of Michigan in 1994 drew attention to the possibility of wildlife being a reservoir host for *M. bovis*. Since 1995, *M. bovis* has been detected in several wildlife and feral species in North America. These species include:

- White-tailed deer (*Odocoileus virginianus*)
- Mule deer (*Odocoileus hemionus*)
- Raccoons (*Procyon lotor*)
- Coyotes (*Canis latrans*)
- Feral cats (*Felis silvestris catus*)
- Black bears (*Ursus americanus*)
- Gray wolves (*Canis lupus*)
- Bobcat (*Lynx rufus*)
- Elk (*Cervus canadensis*)
- Bison (*Bison bison*)
- Moose (*Alces alces*)
- Opossums (*Didelphis virginiana*)
- Gray fox (*Urocyon cinereoargenteus*)
- Feral swine (*Sus scrofa*)
- Red fox (*Vulpes vulpes*)

The species listed above should be considered for incorporation in a surveillance plan if they are located in the area where TB-infected animals are found. Since any mammal may become infected with bovine TB, other species may also be considered for sampling. Some species infected with *M. bovis* are known to serve as a source of reintroduction, while other species have not been identified as a risk either to other wildlife or domestic livestock.

With increased knowledge of the involvement of wildlife in the ecology of bovine TB, there is also a need to better understand the relationship among wildlife, domestic livestock, and bovine TB outbreaks. New detections of bovine TB in domestic herds raise questions concerning the infection status of wildlife in areas containing affected herds and the potential for transmission of bovine TB from infected wildlife to other animals in the area.

### **Resident Wildlife**

Resident wildlife have small home ranges and spend the majority of their time in close proximity to the affected premises.

After trapping resident wildlife on TB-affected premises in Michigan, bovine TB-infected peridomestic resident mammals (e.g. raccoons and opossums) were found on approximately two-thirds of the farms sampled. These multiple-year trapping efforts on affected premises indicate that bovine TB detections in peridomestic mammals are much more likely to occur during the first year of trapping after TB is detected in a domestic herd. Current research does not indicate that peridomestic mammals pose a risk of disease transmission to domestic livestock. However, it is suggested that these animals be removed as part of the cleaning and disinfection effort to reduce the potential for re-infection.

It is recommended that resident mammals (e.g. raccoons and opossums) are trapped and removed as soon as possible after a premises is identified as infected. Removal efforts should continue until no target species are trapped for 5 consecutive nights. Additionally, removal efforts should be conducted at approximately 6-month intervals when trapping conditions are optimal (e.g., spring and fall). Trapping should be conducted for a minimum of 1 year after the last TB positive animal was removed. Removal of peridomestic mammals serve not only to clean and disinfect the premises, but may also offer an opportunity to learn more about the potential role that these animals play in the epidemiology of bovine TB. Testing of these animals is highly recommended.

### **Transient Wildlife**

Transient wildlife are animals that have home ranges generally larger than the size of farms or ranches in the area and, consequently, spend only a portion of their time on the affected premises (e.g., deer, coyotes, and feral swine). These animals may have less direct contact with domestic herds, but due to their increased movements, may have greater potential to spread bovine TB through indirect contact (e.g., contamination of feed) or serve as sentinels for the disease.

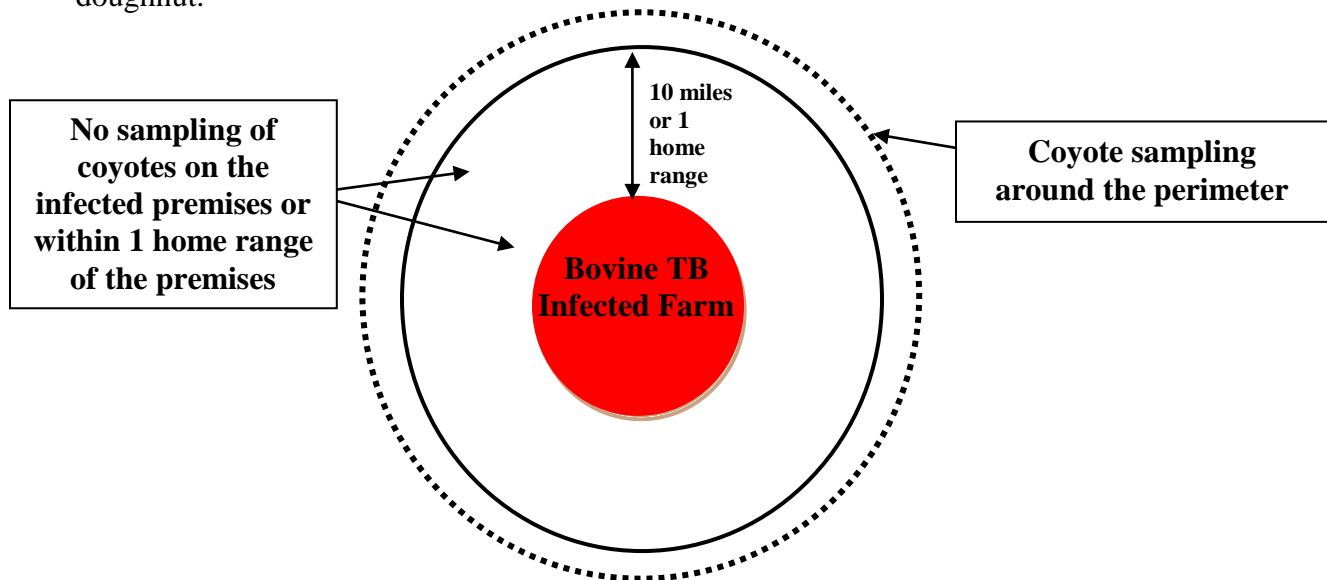
### **Coyotes**

Recently published research indicates that coyotes are good sentinels for bovine TB. Infections primarily have been detected in the mesenteric lymph nodes of coyotes, which indicate that the mycobacterium was ingested. It is recommended that sampling for coyotes begin at least one

home range from the perimeter of the infected premises to distinguish between coyotes that ingested infected livestock on the known infected premises from those that ingested bovine TB from wildlife or infected livestock from neighboring premises. If local coyote home ranges are unknown, surveillance should be conducted beyond an inner radius of 10 miles (i.e., no sampling within 10 miles of the infected premises). Re-evaluation of the survey area may be required if additional affected dairy, beef, or captive cervid herds are identified.

The objective of sampling coyotes is to assist in delineating the extent of bovine TB on the landscape, not to determine if wildlife or livestock are the cause of infection or to estimate the prevalence of disease in target species.

This type of sampling is metaphorically similar to the shape of a doughnut, with the doughnut hole representing the infected premises, and the doughnut or area surrounding the premises representing 1 home range (or 10 miles). Coyote sampling begins around the perimeter of the doughnut.



Coyotes are territorial animals and will move into new territories when other coyotes are displaced. Therefore, samples should be obtained within 6 months to ensure that the information gathered is from coyotes in the surrounding area rather than coyotes from adjacent territories. If possible, samples should be collected from 10 coyotes (at least 1 year old) beyond the perimeter of the infected farm within 6 months of identification of the infected premises.

### **Feral Swine**

Bovine TB has not been detected in the feral swine population in the continental United States, and the epidemiology of bovine TB in countries where *M. bovis* has been identified in feral swine is not fully understood. Feral swine are considered as spillover hosts in some areas and as reservoirs in other areas with high densities. To mitigate the potential threat for bovine TB related to feral swine, it is recommended that efforts be made to remove all feral swine within 10 miles of the premises or a distance determined by local wildlife experts.

## **Cervids**

Telemetry studies conducted in Michigan indicate that a small subset of white-tailed deer account for the majority of visits to farms. Additionally, deer that visit one farm are more likely to visit multiple farms. Cervids are known to become infected and to transmit bovine TB via the oral or respiratory route and are considered reservoirs in some areas. Consequently, samples from cervids are a high priority.

## **Sampling Steps**

The following steps (in no particular order) are recommended within the designated surveillance area:

1. Remove and test deer and feral swine on and within 10 miles of the infected premises, or within a sampling radius determined by local wildlife biologists. The desired sample sizes and time periods for collection will have been determined during the development of a sampling plan. Typically, target samples sizes will be reached within 3 years.
2. Follow up with hunter-harvested surveillance of cervids and feral swine during hunting season to reach the target number of samples.
3. Trap and test target species other than cervids and feral swine within a 1- to 2- mile radius of the infected premises.
4. Trap and test coyotes beyond 1 home range or 10 miles of the infected premises.
5. Trap or otherwise remove and collect samples from small mammals known to be spillover hosts of TB that are present on the premises. The list of species in section II (A) may be used as a reference to identify species of interest. Consult with local wildlife agencies prior to removing any animals to ensure compliance with State and Federal regulations. Begin sampling as soon as possible after detection and continue trapping until 1 year after the last TB-positive animal is removed.

## **Sample Sizes**

**Resident Wildlife:** It may not be necessary to establish a target sample size for peridomestic animals because it is recommended that they be trapped on the infected premises for at least 1 year after removal of the last infected animal. Furthermore, each trapping season will likely result in removal of the entire resident population since trapping will continue until no target species are trapped for at least 5 consecutive nights.

**Transient Wildlife:** The target number of transient animal (e.g., deer) samples may be collected using a combination of hunter-harvested and agency-harvested surveillance efforts on or around the index premises. For agency-harvested surveillance, tissue samples are collected by trained biologists or veterinarians within the designated surveillance area (surrounding the affected premises) shortly after the animal's death. These samples are given more weight (i.e., fewer samples need to be collected) than samples obtained from hunter-harvested surveillance where the entire carcass may not be available for sampling.

## **Sample Size Calculator**

A sample-size calculator has been created based on the epidemiology of bovine TB in Michigan white-tailed deer. This calculator provides one method of determining the number of samples required to detect the presence of bovine TB in deer populations surrounding infected farms.



Other methods or models may also be used to facilitate sample-size determinations. The calculator weights the samples based on age, gender, and collection method and includes a probability of detection. For example, samples from male deer older than 2 years of age are given more weight than samples from deer younger than 2 years of age. Likewise, lymph nodes submitted from throughout the carcass (complete necropsy) of specifically targeted animals are given more weight than hunter-harvested samples (where only the head and associated lymph nodes are available for evaluation). Regardless of the source, all samples are counted and included in the calculations. The probability of detection is defined as the sensitivity of the entire process from sample collection to sample testing.

The target number of samples may be reached over a multiyear period determined during the development of the wildlife surveillance strategy. It is not necessary to collect all of the samples in 1 year. However, the target number of samples should be collected in the least amount of time possible to ensure timely detection of the disease. (The sample calculator is available by contacting any of the first five people listed in Appendix A).

*Note: Caution should be used when applying this sample calculator to elk because the same pattern of weights by age and gender may not apply. Local wildlife biologists should be consulted for population densities and assistance in determining an appropriate sample size for elk.*

### **Selecting Geographic Areas for Sampling**

Resident wildlife should be trapped in the designated surveillance area defined as the infected premises. Since home ranges for cervids and other transient animals vary depending on the region of the country and habitat, the formula below may be used to aid in determining the appropriate geographic area to conduct surveillance. This formula should serve as a starting point and should not be used as the only means for determining the appropriate area to sample. Consideration should be given to the specific species being targeted (e.g., elk versus deer) and the unique ecology of the population being sampled. Additionally, issues related to migratory, nonmigratory, or a population with a combination of migratory and nonmigratory animals should be considered as well as gender and age classes.

*Formula for selecting geographic area for surveillance*

Surveillance radius =  $2 * \sqrt{(A/\pi)}$  where  $A$  is the home range of the species in square miles

For example, if bovine TB is found in an area where deer have a home range of 5 square miles, then  $2 * \sqrt{(5/\pi)} = 2.5$ , meaning that surveillance would be conducted within a 2.5 mile radius around the infected premises or within two home ranges of the infected premises. This formula converts home range in square miles to circular equivalent areas. The diameter of the circular home range then becomes the radius of the surveillance zone. It is important to remember that different species have different size home ranges and the same species may have different size home ranges in different parts of the country.

## **B. Supplies**

### **Sampling Supplies**

The NVSL will provide sampling supplies upon request (see Appendix A for contact information). The supplies needed to sample one animal include:

- Two jars (90 mL) of 10 percent neutral buffered formalin per animal
- Up to three Whirl-Paks® (or other sterile bags) per animal for storing fresh tissues
- Two 4-ounce jars of saturated sodium borate solution per animal (optional for collection from cervids and feral swine)
- Six barcodes per animal: two for the buffered formalin jars (head and chest, abdomen), two for the Whirl-Paks® or sodium borate jars, one for the datasheet, and one for the serum vial (if applicable)
- FedEx shipping label

### **Necropsy Tools**

Suggested necropsy supplies include:

- Sharp knife (hunting knives and slaughterhouse boning knives work well)
- Scissors
- Rat-tooth forceps
- Cutting board
- Personal Protective Equipment (see PPE section below)
- Hack saw or bone saw
- Small and large shears (lopping shears or ratcheted rib cutters for rib cage/sternum)
- Chisel and mallet
- Scalpels and razor blades (disposable scalpels are highly recommended)
- Jaw spreaders for aging cervids and feral swine

### **Blood Collection Supplies**

Blood collection supplies are used only for sampling animals with visible lesions or granulomas.

Those supplies include:

- 1- to 1.5-inch needles (or Vacutainer® needles and holders)
- 3- to 4-inch spinal needles (for intracardiac blood from cervids and feral swine)
- 10 mL syringe
- Vacutainer® tubes
- 4 mL Cryovials®

### **Other Supplies**

Other suggested supplies include:

- Global Positioning System Unit (set to WGS84 and decimal degrees)
- Disinfectant
- Scrub brush
- Large rubber tub (for disinfecting boots and necropsy tools)
- Datasheets
- Plastic bags (large for carcass disposal and small for sample collection)

- Sharpie® or indelible marker (for labeling)
- Pen (for filling out datasheets)
- Plastic sheets, wood chips, pet litter, or other absorbents (for floors in work area)
- Sharps container
- Biohazard waste bag
- Digital camera
- Ruler (for measuring lesions and/or tissue)

## **C. Personal Safety Guidelines and Equipment**

### **Recommendations for Field Biologists and Veterinarians Handling Wildlife**

Bovine TB is zoonotic and presents a risk to human health and safety. Because of this risk, all carcasses should be handled with caution and considered potentially infectious. Precautions for personal safety should be exercised.

Do not eat, drink, or smoke while dissecting a carcass or collecting samples. Establish a clean work zone and a contaminated work zone (clean/dirty line) with an area to disinfect supplies, equipment, and personnel between the two areas. Place datasheets, GPS units, cameras, and other nondisposable equipment in plastic bags or containers that can be disinfected or discarded.

All personnel conducting necropsies or handling animals that may be infected with bovine TB should also have a TB test prior to any potential exposure and annually thereafter (or as recommended by an occupational health professional).

### **Safety and Personal Protective Equipment (PPE)**

Wearing protective gear will minimize the possibility of contact with infectious agents in body fluids and aerosols and reduce the risk of human infection. All necropsy tools and instruments should be disinfected before and between necropsies and after sampling to prevent cross-contamination and infection.

The following PPE are recommended during sample collection from wildlife:

- Heavy-duty disposable gloves (rubber or nitrile)
- Cut-resistant mesh glove on nondominant hand
- Goggles, safety glasses, or face shield
- Disposable apron or an apron that can be disinfected
- Forearm protectors
- Cloth or Tyvek® coveralls
- Rubber boots
- Hair net or hat that can be disinfected
- Respirator (N95 mask at a minimum)

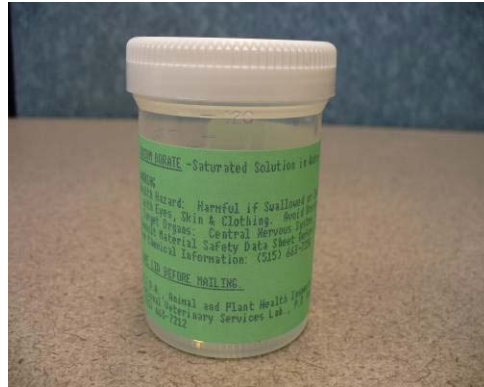
Work upwind of carcasses when performing necropsies outdoors. Always wash hands and exposed skin with soap and warm water or an alcohol-based cleanser after collecting samples.

## Handling Harmful Substances

Sodium borate and 10 percent buffered formalin are hazardous substances that can be inhaled or absorbed through the skin. Carefully handle all harmful substances when sampling and shipping (see Appendices B and C).



**Buffered Formalin**



**Sodium Borate**

Disinfectants are also potentially hazardous and should be handled with care. The Material Safety Data Sheets for each chemical should be reviewed prior to use to ensure that collectors are aware of the dangers associated with handling the disinfectants and chemicals and take the appropriate precautions.

## Carcass Disposal and Disinfectants

The carcass and all tissues from the carcass should be disposed of according to State and local animal carcass disposal regulations. Depending on the State or area, methods may include burial, incineration, composting, or double-bagging and transporting to a landfill. All contaminated paper or plastic materials should be considered hazardous waste and should be thoroughly disinfected, incinerated, or double-bagged and disposed of at the landfill (if permitted).

All blood and tissue should be removed from necropsy instruments and tools with soap and water, rinsed, and subsequently disinfected with an approved disinfectant for bovine TB between necropsies. If performing necropsies in a laboratory setting, a container of 70 percent ethanol or reagent alcohol with sand in the bottom can be used to decontaminate instruments between animals. The excess liquid should be flamed using a Bunsen burner or propane torch. If disinfectants are not thoroughly rinsed (flamed) from the instrument, a false negative result may occur. Conversely, if disinfectants are not used between animals, false positives may be identified.

Gloves should also be changed between animals. Necropsy boots, aprons, and contaminated clothing should be cleaned and thoroughly disinfected upon completion of sample collections. External surfaces of containers with samples should be disinfected.

The products listed in the table below are effective, environmentally friendly disinfectants for use against bovine TB. Additional approved TB disinfectants can be found in the U.S.

Environmental Protection Agency Office of Pesticide Programs List B: EPA’s “Registered Tuberculocide Products Effective Against *Mycobacterium tuberculosis*.”

Disinfectant	Time to Effectively Disinfect	Environmentally Friendly	Manufacturer
Oxivir® Tb	5 minutes	Active ingredients break down to water and oxygen	JohnsonDiversey
Opti-Cide 3®	3 minutes	Contains no dangerous phenols, chlorine, artificial dyes or perfumes	Micro-Scientific Industries
Clorox® Bleach (Mix 1 part bleach with 9 parts water)	5 minutes	Product contains no free chlorine and breaks down into salt and water after use; does not contain dioxins or contaminate groundwater	Clorox® Company

**Disposal of Gloves and Sample-Related Waste**

Spray or soak waste with disinfectant and place in a bag. Spray or soak the bag with disinfectant, place the bag in another bag, and dispose at the landfill (if permitted).

**D. Collecting Specimens**

**General Recommendations**

Despite the stringent decontamination protocols used in the laboratory, tissue specimens can still be overgrown by environmental fungi and bacteria, thereby impeding the ability to recover any viable mycobacteria present in the tissues. To minimize overgrowth, it is important to collect tissues from the animal as soon as possible post mortem. Collect tissues from animals within 2 hours whenever possible. *If the animal has been dead for more than 24 hours, contact the laboratory prior to submitting samples for histopathology.* When performing the necropsy, collect the tissues using aseptic techniques from the head, thoracic cavity, and abdominal cavity (in that order) to minimize cross-contamination.

Species-specific necropsy procedures are provided in the following appendices:

- Appendix D for raccoons
- Appendix E for deer or other cervids
- Appendix F for coyotes
- Appendix G for opossums
- Appendix H for feral swine

**Sample Collection and Storage**

*Collect tissues from animals within 2 hours when possible and ship to NVSL within 72 hours.*

Delays in shipping samples to NVSL may result in false negative results. Extended exposure to sodium borate may make the recovery of *M. bovis* impossible. Extended exposure to 10 percent buffered formalin will denature the proteins and render the samples unsuitable for testing.

Samples submitted for histopathology should be placed in 10 percent buffered formalin.

Samples submitted for culture should be collected in Whirl-Paks® and kept cold until shipped,

or in sodium borate at room temperature. Tissues from the head and chest can be combined in the same Whirl-Pak® or jar. However, lymph nodes or tissues from the abdominal region should be placed in separate containers to prevent contamination. Fresh, well preserved samples increase the probability of culturing and detection of *M. bovis*.

### **Samples to Collect**

Collect submandibular, retropharyngeal, tracheobronchial, mediastinal, and mesenteric lymph nodes from each animal, as well as a section of tonsils, lungs, and any tissues with gross lesions. Carefully examine the lungs and palpitate for abnormalities. Collect parotid lymph nodes from cervids and feral swine. The collection of mesenteric lymph nodes from non-cervids is especially important because lesions are often found in these nodes. Blood should also be collected from animals exhibiting gross lesions suggestive of bovine TB infection.

### **When to Use Formalin, Whirl-Paks® and/or Sodium Borate**

Samples submitted for culture should be placed in Whirl-Paks®. Samples submitted for histopathology should be placed in 10 percent buffered formalin jars. Lymph nodes and tissues collected from cervids and feral swine can be placed in sodium borate jars for culture analysis if preferred by the collector. An advantage of sodium borate is that the tissue can be preserved without refrigeration and it decreases the risk of tissue contamination by other bacteria. However, if the sample does not arrive at the laboratory for culture testing within 72 hours, the sodium borate may penetrate the tissue sample and kill any mycobacteria that may be present, thereby increasing the risk of a false negative culture result. Tissues from species other than cervids or feral swine are too small (less than 1 inch in diameter) to be placed in sodium borate because the borate will penetrate the tissue too quickly and kill any mycobacteria present.

Use a 10:1 formalin to tissue ratio when submitting samples for histopathology. Tissues placed in formalin should be approximately 3/8-inch or less (e.g., the width of a pencil). For larger tissues, cut 1/4-inch sections to place in the formalin. Use equal parts of tissue and sodium borate (if appropriate) when submitting for mycobacteriologic culture. Tissues submitted in Whirl-Paks® should be refrigerated or placed in a cooler with ice until shipped to the laboratory. If fresh tissues for culture will be held more than 72 hours prior to shipping, freeze at -20°C and ship the tissues frozen on ice packs.

<b>Test</b>	<b>Species</b>	<b>Tissue collection</b>
Histopathology	All	Formalin
Culture	All	Whirl-Paks®
Culture (optional)	Cervids & Feral Swine	Sodium borate

### **Head Lymph Nodes and Tissues**

The retropharyngeal, submandibular, and parotid lymph nodes and tonsils are targeted for sampling. However, the parotids are difficult to locate in small mammals; therefore, it is not necessary to submit parotids from non-cervids (other than feral swine). Lymph nodes in the head and neck region are paired. Submit both parotid, submandibular, and retropharyngeal lymph nodes from each animal when possible. For non-cervids, collect one of each lymph node for culture and the other lymph node for histopathology. For cervids and feral swine, lymph nodes must be sectioned before placing the samples in formalin. Place a 3/8-inch section (width of a

pencil) of each lymph node in formalin and keep the remainder of each for culture submission. If both lymph nodes cannot be recovered from each animal, submit one for histopathology screening. When possible, extract tonsils to submit for testing (one for culture, and one for histopathology).

### **Thoracic Lymph Nodes and Tissues**

All tissues in the chest should be examined thoroughly for evidence of TB infection (i.e., lesions). Lymph nodes from the chest cavity include the mediastinal lymph node (chain of nodes; typically found between the aorta and lungs and near the heart) and tracheobronchial lymph nodes (paired and found where the trachea splits into the main bronchi on each side). Due to the small size of these lymph nodes in species other than cervids, collect and submit these lymph nodes for culture only. Carefully examine the lungs and submit a subsection of at least two lobes of the lung.

### **Abdominal Cavity Lymph Nodes**

The mesenteric lymph nodes are the only lymph nodes to routinely submit from the abdominal cavity. They are located in the mesentery associated with the bowel, usually most obvious between the loops of small intestine where the blood vessels converge. Placement and size of the lymph nodes can vary depending on species. Carefully examine the mesenteric lymph nodes of non-cervids because lesions in these lymph nodes are more common in non-cervids than in cervids. For species with mesenteric lymph nodes greater than  $\frac{3}{8}$ -inch thick (width of a pencil), place  $\frac{3}{8}$ -inch sections in formalin for histopathology and keep the remainder for culture.

### **Collection of Lesions or Abnormal Tissue**

All observed lesion(s) should be submitted in separate containers for laboratory analysis. When multiple lesions are found in the same tissue, submit a small section (the size of a golf ball) for histopathology and culture and include sections of adjacent normal tissue. If only one lesion is found, divide the tissue in half through the middle of the lesion and submit half for histopathology and half for culture (include adjacent normal tissue). If there are insufficient lesions to divide, submit the available tissue for culture testing only (see Appendix I).

When sampling, carefully examine the chest cavity and lungs for the presence of lesions and/or granulomas. Be sure to feel and visually inspect each lung lobe carefully (see Appendix I).

*IMPORTANT: Collect tissues with lesions in a separate container. Do not include lesions in the same containers with regularly collected lymph nodes or tissues, or in a container with other lesioned tissue samples. Label the container (not the lid) with the location where lesions were found. When submitting tissue with lesions, be sure to document the following in the comments section of the datasheet: the origin of the tissue affected, number of lesions, distribution and pattern, size, shape, color, consistency and texture of the lesion. Additionally, submit a digital photograph of all of the lesions with an adjacent object that indicates the scale of the lesion, if possible.*

## Tissue Selection and Preservation

1. Always clean and disinfect instruments between necropsies of each animal. If disposable scalpels are available, discard them after each animal. Thoroughly rinse instruments after disinfecting to ensure that the samples are not inadvertently disinfected. Change gloves between the necropsy of each animal.
2. Submit lymph nodes in separate containers based on the region of the body from which they are collected (head and chest combined, and abdomen in a separate container). Use two formalin jars and two Whirl-Paks® (or optional two sodium borate jars for cervids or feral swine) for all other species if no gross lesions are observed. *Note: Tissues with lesions should be submitted in separate containers for culture and labeled with the tissue type in addition to the barcode.*
3. Label the Whirl-Paks® with the last four digits of the barcode and “HC” or “A” to correspond to the head and chest or abdomen (see picture below). After the samples have been collected, place all Whirl-Paks® in a larger Ziploc® bag with the barcode affixed to the Ziploc® bag.



4. Assign a unique barcode to each animal. Apply a barcode with the same number to the formalin jars, the Ziploc® bag (with Whirl-Paks® inside), the serum vial, and the datasheet. *Tissues from different animals should never be combined in the same jars or Whirl-Paks®.*
5. For samples submitted for histopathology, divide the tissues or lymph nodes into slices approximately  $\frac{3}{8}$ -inch thick (width of a pencil). Place lymph nodes or tissues in the formalin jar. If lesions are observed, collect sections of the lesions and include normal tissues surrounding the lesions. Lymph nodes submitted for histopathology should be cut in half to ensure that they become formalin-fixed (unless they are the size of a jelly bean or smaller).
6. For small lymph nodes, do *not* cut lymph nodes in half when submitting for culture. Store fresh tissues in Whirl-Paks® and then place the samples on ice. For larger ( $>\frac{3}{8}$ -inch thick) lymph nodes, cut  $\frac{3}{8}$ -inch sections for formalin and submit the remainder for culture. For collection of cervid samples using sodium borate, cut a 1-inch cube of tissue (approximately the size of a golf ball) and place the lymph node or tissue into the sodium borate jar. *Tissues less than 1-inch thick should not be placed in sodium borate.*
7. If the sample volume is insufficient to divide for histopathology and culture, it is recommended that samples are submitted for culture if gross lesions are present; or samples are submitted for histopathology if gross lesions are not present.



8. After sample collection, disinfect the outside of each Whirl-Pak® or container in an approved disinfectant for TB. Use caution to ensure that no disinfectant enters the Whirl-Pak® or container. Remember to keep the surface wet with disinfectant solution for the required contact time (see the table on page 13 or refer to the product label). Rinse with water after the contact time requirement has been met.
9. Tighten the caps on specimen containers and seal with Parafilm®. Electrical tape also can be used if Parafilm® is not available. Place a barcode on the outside of the specimen



container. The Whirl-Paks® for culture and the formalin jars for histopathology will receive the same number because they are from the same animal.

10. Do not freeze specimens that will be submitted for histopathology. Fresh tissues for culture submitted in Whirl-Paks® should be kept cool on ice or refrigerated until shipped to the laboratory. If fresh tissues will be held more than 3 days before shipping, freeze at -20°C and ship frozen tissues on ice packs. Formalin fixed tissues as well as fresh tissues stored in sodium borate can be kept at room temperature until shipped.
11. Samples should be shipped to the NVSL within 24 hours of collection when possible. Avoid weekend delivery unless prior arrangements have been made with the laboratory staff (see shipping section below for instructions and address).

Species	Region	Samples Collected	Storage
Cervids and Feral Swine	Head	retropharyngeal, submandibular, parotid, tonsils	Whirl-Paks® for culture (sodium borate optional) and formalin for histopathology
	Chest	mediastinal, tracheobronchial, lung	
	Abdomen	mesenteric	
	Lesions	any tissue with lesions	
Non-cervids (Raccoons, Opossums, and Coyotes)	Head	retropharyngeal, submandibular, tonsils	Whirl-Paks® for culture and formalin for histopathology
	Chest	mediastinal, tracheobronchial, lung	
	Abdomen	mesenteric	
	Lesions	any tissue with lesions	

## **Blood Collection**

*Blood should only be collected from wildlife with visible lesions or granulomas.* If bovine TB or TB-like granulomas or lesions are observed anywhere in the carcass, use a needle and syringe to extract as much blood as possible from the heart.

1. Place extracted blood into a 10 mL red-top or serum-separator tube (collect at least two tubes if possible).
2. Blood should be left undisturbed for approximately 30 minutes at room temperature to encourage clot formation prior to centrifugation.
3. Centrifuge for 10 minutes at a minimum of 1,800 revolutions per minute for 15 minutes to separate the serum from the blood cells. Then use a sterile disposable pipette to transfer the serum to a polypropylene Cryovial® (or pour serum off if using a serum-separator tube). If a centrifuge is not available, serum can be obtained by letting the clot or blood cells settle, then chill in refrigerator to contract the clot, and transfer the serum to a polypropylene Cryovial®.
4. Submit at least 1 mL of serum to the TB serum bank. Transfer the serum to a single polypropylene Cryovial® and refrigerate, or transfer 0.5 mL of serum into 1.2 mL polypropylene Cryovials® and freeze at -20°C.
5. Label the serum vial with the sample barcode number using a Sharpie® or permanent marker.
6. Refrigerated serum should be shipped to NVSL with ice packs within 2 weeks of collection.
7. Frozen serum can be shipped to NVSL once a month on dry ice to ensure the samples remain frozen during shipping. *Do not freeze serum samples that contain more than 0.5 mL of serum.* This will reduce the number of freeze-thaw cycles during processing at NVSL to ensure that sample quality is maintained.

Submit all serum to:  
National Veterinary Services Laboratories  
Attention: Jeff Nelson, TB Serum Bank  
1920 Dayton Avenue  
Ames, Iowa 50010

Questions regarding serum collection or handling procedures should be directed to Jeff Nelson by e-mail at [Jeffrey.T.Nelson@aphis.usda.gov](mailto:Jeffrey.T.Nelson@aphis.usda.gov) or by telephone at (515) 337-7966.

## **Proper Labeling of Samples**

All samples collected from the same animal should be labeled with the same unique barcode—including serum samples. This label should be placed on the container (not on the lid). If fresh tissues are submitted in Whirl-Paks®, at least the last four digits of the barcode number should be written with a Sharpie® or permanent marker. All of the individual Whirl-Paks® should be stored in a larger Ziploc® bag identified with the sample barcode. Place the samples stored in Whirl-Paks® in a cooler and/or on ice packs, or refrigerate immediately.

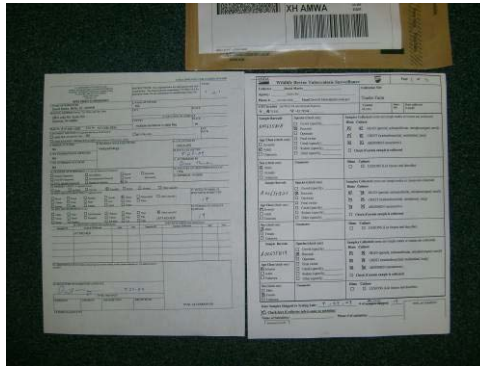
## **Instructions for Wildlife Bovine Tuberculosis Surveillance Datasheets**

1. Record the collector's name, agency, phone number, and e-mail address in the upper left corner.
2. Record the collection site name. A collection site is defined as the refuge, lake, property name, dairy name or other name used by the collector to identify the location. Be as specific as possible. The producer's farm name also can be listed as the collection site if samples are collected on a dairy or beef operation.
3. Collect the GPS coordinates for the site using a GPS unit set to datum WGS 84 and decimal degrees. The site is defined as the general location where the samples are collected. For example, the site may be the front gate of the infected premises, the reservoir, or refuge. Collect one set of coordinates per site.
4. Record the county and State where the samples are collected and the date collected in the corresponding boxes. The date should be recorded using the month, day, year format (mm/dd/yyyy).
5. Samples collected from each animal should be assigned a unique barcode. Place one barcode on the datasheet, one on each jar of formalin, one on each Ziploc® (with two Whirl-Paks® inside), and one on the Cryovial® of serum (if applicable).
6. Check the box next to the species from which the sample is being collected. If the species is a cervid or canid, specify which animal is being tested in the space next to the box. Specify the species being tested if the animal is not listed on the datasheet.
7. Check the box corresponding to the age, class, and sex for each animal.
8. Specify whether the samples are being submitted for histopathology (in formalin) and/or culture (in Whirl-Paks® or sodium borate) by checking the box next to the region of the body.
9. Check the corresponding box if no gross lesions are observed.
10. If any tissues with lesions are collected, specify which tissue is being submitted and describe the lesions in the appropriate box. Send digital photographs by e-mail to the appropriate contact person (see Communication When Shipping Samples to NVSL). Label any digital photographs with the sample barcode of the animal.
11. At the bottom of the datasheet, indicate the date the samples are shipped to NVSL and the total number of sample containers that are included in the shipment (regardless of species).
12. The submitter is defined as the person responsible for shipping the samples to NVSL for testing. If the shipper and the collector are the same, check the box. If not, mark the name of the shipper and provide the appropriate telephone number.

## **Shipping Samples to NVSL**

Send all of the formalin-fixed and fresh tissues (or sodium borate) for each animal in a single shipping container. NVSL will assign an accession number to each shipment and will keep the samples together to ensure that the results are reported under the same accession number.

1. Seal a copy of the completed Wildlife Bovine Tuberculosis Surveillance Datasheet (see Appendix J) and a copy of the VS 10-4 form (see Appendix K) in a plastic bag and place them between the Styrofoam™ cooler and the cardboard box. Do not place datasheets inside the Styrofoam™ cooler.
2. Seal the box with packing tape.



3. *Samples must be shipped Monday, Tuesday, Wednesday or Thursday of each week to ensure arrival at the laboratory on a weekday.*
4. Contact the laboratory prior to shipment and provide the number of samples submitted for histopathology and culture and the FedEx tracking number (see Appendix A for specific laboratory contact information).
5. All samples should be shipped FedEx priority overnight using the prepaid FedEx label provided by NVSL to:

U.S. Department of Agriculture  
Animal and Plant Health Inspection Service  
National Veterinary Services Laboratories  
TB Wildlife Surveillance Samples  
1920 Dayton Road  
Ames, Iowa 50010

### **Communication When Shipping Samples to NVSL**

Contact the laboratory prior to shipping (see Appendix A). Notify the Mycobacteriology Laboratory when sending samples for culture by telephone at (515) 337-7388 or by e-mail at [Suelee.Robbe-Austerman@aphis.usda.gov](mailto:Suelee.Robbe-Austerman@aphis.usda.gov) or [Doris.M.Bravo@aphis.usda.gov](mailto:Doris.M.Bravo@aphis.usda.gov). Histopathology Laboratory staff may be contacted by telephone at (515) 337-7521 or by e-mail at [Bruce.V.Thomsen@aphis.usda.gov](mailto:Bruce.V.Thomsen@aphis.usda.gov) or [Mark.Hall@aphis.usda.gov](mailto:Mark.Hall@aphis.usda.gov). For serum samples, notify Jeff Nelson by telephone at (515) 337-7966 or by e-mail at [Jeffrey.T.Nelson@aphis.usda.gov](mailto:Jeffrey.T.Nelson@aphis.usda.gov).

### **Reporting Results**

Results will be reported directly to the submitter by e-mail. The submitter is responsible for either sending an updated spreadsheet to Wildlife Services' National Wildlife Disease Program in Fort Collins, Colorado (contact [Kerri.Pedersen@aphis.usda.gov](mailto:Kerri.Pedersen@aphis.usda.gov) for an updated version of the spreadsheet) or forwarding the datasheets and results reports for inclusion in the national database to [wslabresults@aphis.usda.gov](mailto:wslabresults@aphis.usda.gov).

## **III. Testing of Samples**

### **A. Histopathology**

Histopathology is a rapid method of identifying disease processes occurring in the tissue and typically takes 1 to 3 days to complete. If the microscopic lesion consists of granulomas that

contain acid-fast bacteria, the case is diagnosed as mycobacteriosis compatible. This diagnosis means the lesion is consistent with tuberculosis; however, the species of acid-fast bacteria causing this lesion cannot be determined using histopathology alone. A diagnosis of mycobacteriosis compatible is not a diagnosis of infection with *M. bovis*.

The process of testing the samples involves technicians examining and cutting each tissue to a 2 mm thick (quarter-sized) subsample. Formalin changes the color and texture of tissues; therefore, it is critical that the collector record any lesions on the “Wildlife Bovine Tuberculosis Surveillance Datasheet.” Detailed descriptions of lesions will assist the laboratory technician in identifying and sampling any small lesions that may be obscured by the formalin fixation. Subsamples are collected and further processed, stained, and mounted onto slides that are examined by pathologists the following day.

## **B. Polymerase Chain Reaction**

Polymerase chain reaction (PCR) testing is conducted only if histopathology indicates the presence of acid-fast bacteria and typically takes 3 to 8 days for results after histopathology testing has been completed. This test is conducted on formalin fixed tissues. *Tissues that remain in formalin for more than 7 to 10 days before being processed may result in false negative test results due to denaturing of the DNA; therefore, timely submission of samples to the laboratory is important.*

PCR is used to determine the presence of genetic material from the *Mycobacterium tuberculosis* complex (which includes *M. bovis*, *M. tuberculosis*, and several other species), *M. avium* and *M. a. paratuberculosis*. However, PCR does not detect all of the other species of mycobacteria. Additionally, a negative finding by PCR does not necessarily mean absence of *M. bovis* and a positive result does not necessarily mean the animal was infectious or shedding.

## **C. Mycobacteriologic Culture**

Fresh tissues submitted for culture of mycobacteria are first screened for visible lesions in the laboratory. Tissues with lesions are processed separately from nonlesioned tissues. If no lesions are observed, submitted tissues from each animal are pooled using a representative section of each tissue submitted.

Tissues are decontaminated with a sodium hydroxide solution to remove fungal and bacterial contaminants, followed by inoculation of various solid and liquid culture media to recover any mycobacterial species that may be present. *M. bovis* is a slow-growing microorganism that usually takes 4 to 8 weeks to grow. Culture media is monitored weekly for a total of 8 weeks to detect potential positive cases. An acid-fast stain is performed if suspicious growth is noted on any media during the 8 week incubation period. If acid-fast bacteria are observed, a GenProbe test for *M. tuberculosis* complex is ordered with results available within 3 to 5 days. This GenProbe test is a commercially available test kit that identifies the unknown bacteria as either positive or negative for the *M. tuberculosis* complex. Since *M. tuberculosis* complex contains multiple species (*M. tuberculosis*, *M. bovis*, and several others), further testing is needed on all

GenProbe positive cases to identify the acid-fast bacteria as *M. bovis*. This confirmatory testing, called spoligotyping, usually takes an additional 3 to 7 days to complete.

If acid-fast bacteria other than *M. tuberculosis* complex are recovered, the result “acid-fast bacteria recovered, not *M. tuberculosis* complex” is reported. Definitive identification of these acid-fast bacteria is usually not performed unless prior arrangements have been made with the laboratory. In these cases, an additional 4 to 8 weeks may be required to identify atypical mycobacteria to the species level.

## **IV. Data Management**

### **A. Overview**

All data should be reported to WS’ National Wildlife Disease Program by e-mail to [nwdpdata@aphis.usda.gov](mailto:nwdpdata@aphis.usda.gov). This type of centralized data storage will facilitate identification of patterns and can be used to generate reports, graphs, and maps. The centralized data storage will also serve as a resource for providing information to the public and to inform policy makers about activities related to TB surveillance in wildlife.

### **B. Data Entry and Responsibilities**

The collector is responsible for completing all of the information on the datasheet (see Appendix J). Collectors should enter their data into the “Tuberculosis in Wildlife Data Collection” spreadsheet and then e-mail it to [nwdpdata@aphis.usda.gov](mailto:nwdpdata@aphis.usda.gov) when samples have been submitted to NVSL for testing.

### **C. Data Mapping and Reporting**

Results reports should be forwarded to [nwdpdata@aphis.usda.gov](mailto:nwdpdata@aphis.usda.gov) for inclusion in the national database. Maps will be available to collectors upon request.

## **Acknowledgements**

*Guidelines for Surveillance of Bovine Tuberculosis in Wildlife* was developed in collaboration between APHIS’ WS and VS. We would like to extend our appreciation to the Michigan Department of Natural Resources and Environment, the Minnesota Department of Natural Resources, and USDA’s Agricultural Research Service for reviewing the manual and providing consultation and photographs of lesions.

**Appendix A: National Wildlife TB Surveillance Contacts**

**National Wildlife TB Surveillance Contacts**

<b>Organization Office</b>	<b>Contact Name</b>	<b>Phone</b>	<b>E-mail</b>
USDA, APHIS, WS, National Wildlife Disease Program	Tom DeLiberto	(970) 266-6088	<a href="mailto:Thomas.J.DeLiberto@aphis.usda.gov">Thomas.J.DeLiberto@aphis.usda.gov</a>
	Tom Gidlewski	(970) 266-6350	<a href="mailto:Thomas.Gidlewski@aphis.usda.gov">Thomas.Gidlewski@aphis.usda.gov</a>
	Kerri Pedersen	(970) 266-6272	<a href="mailto:Kerri.Pedersen@aphis.usda.gov">Kerri.Pedersen@aphis.usda.gov</a>
USDA, APHIS, VS	Bill Hench	(970) 494-7378	<a href="mailto:Charles.W.Hench@aphis.usda.gov">Charles.W.Hench@aphis.usda.gov</a>
	Kathy Orloski	(970) 494-7221	<a href="mailto:Kathy.A.Orloski@aphis.usda.gov">Kathy.A.Orloski@aphis.usda.gov</a>
	Alecia Naugle	(301) 734-7569	<a href="mailto:Alecia.L.Naugle@aphis.usda.gov">Alecia.L.Naugle@aphis.usda.gov</a>
USDA, APHIS, VS Mycobacteriologic Culture	Suelee Robbe-Austerman	(515) 337-7388	<a href="mailto:Suelee.Robbe-Austerman@aphis.usda.gov">Suelee.Robbe-Austerman@aphis.usda.gov</a>
USDA, APHIS, VS, Histopathology & PCR	Bruce Thomsen	(515) 337-7521	<a href="mailto:Bruce.V.Thomsen@aphis.usda.gov">Bruce.V.Thomsen@aphis.usda.gov</a>
	Mark Hall	(515) 337-7521	<a href="mailto:Mark.Hall@aphis.usda.gov">Mark.Hall@aphis.usda.gov</a>
USDA, APHIS, VS, Serum Bank	Jeff Nelson	(515) 337-7966	<a href="mailto:Jeffrey.T.Nelson@aphis.usda.gov">Jeffrey.T.Nelson@aphis.usda.gov</a>
USDA, APHIS, VS, Sampling Supplies	Jason Bunn	(515) 337-7530	<a href="mailto:Jason.K.Bunn@aphis.usda.gov">Jason.K.Bunn@aphis.usda.gov</a>
	Lorie Walsh	(515) 337-7530	<a href="mailto:Lorie.K.Walsh@aphis.usda.gov">Lorie.K.Walsh@aphis.usda.gov</a>

**MSDS****Material Safety Data Sheet**

337 Gordon Drive • Exton, PA 19341 • (610) 524-5810

24 HOUR EMERGENCY  
TELEPHONE NUMBER  
CALL CHEMTREC  
1-800-424-9300

### FORMALIN SOLUTION 10% Neutral Buffered MATERIAL SAFETY DATA SHEET

Date Issue:

The following information is believed to be correct but is not warranted as such, nor does it purport to be all inclusive.

Product Identification				
Product Name:	10% Neutral Buffered Formalin			
Product Code:	PFNBF-180, PFNBF-240, PFNBF-360, PFNBF-1000			
Product Description:	A buffered aqueous solution of formaldehyde and methanol			
	Health	2		
	Flammability	0		
	Reactivity	0		
	Physical Hazard	None		
Section 1 - Shipping Data				
DOT Shipping Name:*	Ground shipments Not regulated	Tel. # for information:	(610) 524-5810	
DOT Hazard Class:	Not applicable	Emergency Tel. #:	(800) 424-9300	
DOT Identification:	Not applicable	Prepared by:	P.B.	
Section 2 - Hazardous Ingredients / Identity Information				
CHEMICAL COMPONENTS	CAS#	%	OSHA PEL	ACGIH TLV
Formaldehyde	50-00-0	3-4%	0.75 ppm	1 ppm STEL
Methyl Alcohol	67-56-1	~1%	200 ppm TWA	200 ppm STEL
Section 3 - Physical / Chemical Characteristics				
Boiling Point:	~100° C	Specific Gravity (H <sub>2</sub> O = 1):	1.01	
Vapor Pressure (mm Hg and Temperature):	18@20°C	Evaporation Rate ( <i>n-butyl alcohol</i> = 1):	1	
Vapor Density (AIR=1):	1	Solubility in Water:	Complete	
Appearance and Odor:	Clear, colorless solution. Characteristic odor of formaldehyde.			
Section 4 - Fire and Explosion Hazard Data				



## Appendix B: MSDS for 10% Buffered Formalin

**Flash Point (Method Used):** Not applicable                      **Flammability Limits:** Not applicable  
**Extinguishing Media:** Dry Chemical, carbon dioxide or alcohol type foam.  
**Special Fire Fighting Procedures:** Use self-contained breathing apparatus and full protective clothing.  
**Unusual Fire and Explosive Hazards:** Pyrolysis will release toxic compounds such as carbon monoxide and formaldehyde.

### Section 5 - Reactivity Data

**Stability:** Polymerizes slowly to form paraformaldehyde.                      **Conditions to Avoid:** Heat.  
**Incompatibility (Materials to Avoid):** Strong oxidizers, strong alkalis, acids, phenol and urea.  
**Precautions to be taken in Handling and Storage:** Store at room temperature. Do not freeze.

### Section 6 - Health Hazard Data

<b>Routes of Entry</b>	<b>Inhalation?</b> yes	<b>Skin Absorption?</b> yes	<b>Ingestion?</b> yes
<b>Carcinogenicity?</b> yes	<b>NTP?</b> no	<b>IARC Monographs?</b> yes	<b>OSHA Regulated?</b> yes

**Health Hazards (Acute and Chronic):** . Inhalation can lead to congestion, coughing and shortness of breath. Frequent skin contact leads to drying and scaling. Ingestion will damage the throat, stomach and intestines resulting in nausea, vomiting, abdominal pain and diarrhea. Lowered blood pressure, spontaneous abortion, loss of consciousness and kidney damage may result. Inhalation of high concentrations of vapor (14 ppm) have caused cancer in laboratory animals. Genetic damage in bacteria has been demonstrated.

**Medical Conditions Generally Aggravated by Exposure:** People who regularly work with formaldehyde are required to have regular medical surveillance.

**Emergency and First Aid Procedures:** *Seek medical assistance for further treatment, observation and support if necessary.*

**Eye Contact:** Irrigate immediately with large quantity of water for at least 15 minutes. Get medical attention immediately.

**Skin Contact:** Remove contaminated clothing and flush with water.

**Ingestion:** Dilute immediately with water or milk. Induce vomiting followed by a slurry of activated charcoal.. Do not induce vomiting if patient is unconscious or drowsy, contact physician.

**Inhalation:** Remove to fresh air. Give artificial respiration if necessary. Contact physician.

**All other means of exposure:** Contact poison control center immediately. Be prepared to provide hazardous ingredient information.

### Section 7 - Precautions For Safe Handling and Use

**Steps to be Taken In Case of Spill Or Release:** Absorb with a suitable absorbent (such as paper towel) and dispose.

**Waste Disposal Methods:** The disposal of formaldehyde is usually restricted. Incineration is the preferred method of disposal. Waste water disposal permits sometimes allow small quantities to be flushed down drain with excess water. Ensure compliance with all government regulations.

### Section 8- Control Measures

**Respiratory Protection (Specify Type):** Recommended.

**Ventilation:** Use local mechanical exhaust such as a chemical fume hood.

**Protective Gloves:** Use rubber or plastic gloves that are impervious to formaldehyde.

**Eye Protection:** Laboratory safety goggles or similar products are recommended as part of good laboratory practice.

**Other Protective Clothing And Equipment:** Use chemical resistant clothing.

**Hygienic Work Practices:** Wash well after handling, especially before eating and smoking.

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# SIGMA-ALDRICH

[sigma-aldrich.com](http://sigma-aldrich.com)

## Material Safety Data Sheet

Version 4.0  
Revision Date 03/12/2010  
Print Date 07/06/2010

### 1. PRODUCT AND COMPANY IDENTIFICATION

Product name : Sodium tetraborate decahydrate

Product Number : S9640

Brand : Sigma-Aldrich

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +18003255832

Fax : +18003255052

Emergency Phone # : (314) 776-6555

### 2. HAZARDS IDENTIFICATION

#### Emergency Overview

#### OSHA Hazards

Teratogen, Reproductive hazard

#### GHS Label elements, including precautionary statements

Pictogram



Signal word

Danger

Hazard statement(s)

H303

May be harmful if swallowed.

H360

May damage fertility or the unborn child.

Precautionary statement(s)

P201

Obtain special instructions before use.

P308 + P313

IF exposed or concerned: Get medical advice/attention.

#### HMIS Classification

Health hazard: 1

Chronic Health Hazard: \*

Flammability: 0

Physical hazards: 0

#### NFPA Rating

Health hazard: 0

Fire: 0

Reactivity Hazard: 0

#### Potential Health Effects

Inhalation : May be harmful if inhaled. May cause respiratory tract irritation.

Skin : May be harmful if absorbed through skin. May cause skin irritation.

Eyes : May cause eye irritation.

Ingestion : May be harmful if swallowed.

### 3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Boraxdecahydrate  
Sodium boratedecahydrate

## Appendix C: MSDS for Sodium Borate

Formula :  $B_4Na_2O_7 \cdot 10H_2O$   
Molecular Weight : 381.37 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
Disodium tetraborate decahydrate			
1303-96-4	215-540-4	005-011-01-1	-

### 4. FIRST AID MEASURES

#### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

#### If inhaled

If breathed in, move person into fresh air. If not breathing give artificial respiration. Consult a physician.

#### In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

#### In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

#### If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

### 5. FIRE-FIGHTING MEASURES

#### Suitable extinguishing media

Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.

#### Special protective equipment for fire-fighters

Wear self contained breathing apparatus for fire fighting if necessary.

#### Further information

The product itself does not burn.

### 6. ACCIDENTAL RELEASE MEASURES

#### Personal precautions

Use personal protective equipment. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Evacuate personnel to safe areas.

#### Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

#### Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

### 7. HANDLING AND STORAGE

#### Precautions for safe handling

Avoid formation of dust and aerosols.

Provide appropriate exhaust ventilation at places where dust is formed.

#### Conditions for safe storage

Keep container tightly closed in a dry and well-ventilated place.

### 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

#### Components with workplace control parameters

Components	CAS-No.	Value	Control parameters	Update	Basis
Disodium	1303-96-4	TWA	2 mg/m <sup>3</sup>	2005-01-01	USA, ACGIH Threshold Limit Values

## Appendix C: MSDS for Sodium Borate

tetraborate decahydrate					(TLV)
Remarks	<p>Not classifiable as a human carcinogen: Agents which cause concern that they could be carcinogenic for humans but which cannot be assessed conclusively because of a lack of data. In vitro or animal studies do not provide indications of carcinogenicity which are sufficient to classify the agent into one of the other categories.</p> <p>Inhalable fraction. See Appendix C, paragraph A. Inhalable Particulate Mass TLVs (IPM-TLVs) for those materials that are hazardous when deposited anywhere in the respiratory tract.</p> <p>ACGIH 2005 Adoption Refers to Appendix A -- Carcinogens.</p>				
		STEL	6 mg/m <sup>3</sup>	2005-01-01	USA. ACGIH Threshold Limit Values (TLV)
	<p>Not classifiable as a human carcinogen: Agents which cause concern that they could be carcinogenic for humans but which cannot be assessed conclusively because of a lack of data. In vitro or animal studies do not provide indications of carcinogenicity which are sufficient to classify the agent into one of the other categories.</p> <p>Inhalable fraction. See Appendix C, paragraph A. Inhalable Particulate Mass TLVs (IPM-TLVs) for those materials that are hazardous when deposited anywhere in the respiratory tract.</p> <p>ACGIH 2005 Adoption Refers to Appendix A -- Carcinogens.</p>				

### Personal protective equipment

#### Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

#### Hand protection

Handle with gloves.

#### Eye protection

Safety glasses with side-shields conforming to EN166

#### Skin and body protection

Choose body protection according to the amount and concentration of the dangerous substance at the work place.

#### Hygiene measures

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### Appearance

Form	crystalline
Colour	white

### Safety data

pH	9.2 at 10 g/l
Melting point	62 °C (144 °F)
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Density	1.73 g/mL at 25 °C (77 °F)
Water solubility	38.1 g/l at 20 °C (68 °F) - completely soluble

## Appendix C: MSDS for Sodium Borate

### 10. STABILITY AND REACTIVITY

**Chemical stability**

Stable under recommended storage conditions.

**Conditions to avoid**

no data available

**Materials to avoid**

Strong oxidizing agents, Strong reducing agents

**Hazardous decomposition products**

Hazardous decomposition products formed under fire conditions. - Borane/boron oxides, Sodium oxides

---

### 11. TOXICOLOGICAL INFORMATION

**Acute toxicity**

LD50 Oral - rat - 4,500 - 5,000 mg/kg

LD50 Dermal - rabbit - 10,000 mg/kg

**Skin corrosion/irritation**

no data available

**Serious eye damage/eye irritation**

no data available

**Respiratory or skin sensitization**

no data available

**Germ cell mutagenicity**

no data available

**Carcinogenicity**

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

fetotoxicity

Presumed human reproductive toxicant

Presumed human reproductive toxicant

**Specific target organ toxicity - single exposure (GHS)**

no data available

**Specific target organ toxicity - repeated exposure (GHS)**

no data available

**Aspiration hazard**

no data available

**Potential health effects****Inhalation**

May be harmful if inhaled. May cause respiratory tract irritation.

**Ingestion**

May be harmful if swallowed.

**Skin**

May be harmful if absorbed through skin. May cause skin irritation.

**Eyes**

May cause eye irritation.

## Appendix C: MSDS for Sodium Borate

### Signs and Symptoms of Exposure

Animal feeding studies in rat, mouse and dog, at high doses, have demonstrated effects on fertility and testes. Studies with the chemically related boric acid in the rat, mouse and rabbit, at high doses, demonstrate developmental effects on the fetus, including fetal weight loss and minor skeletal variations. The doses administered were many times in excess of those to which humans would normally be exposed. Human epidemiological studies show no increase in pulmonary disease in occupational populations with chronic exposures to boric acid dust and sodium borate dust. A recent epidemiological study under the conditions of normal occupational exposure to borate dusts indicated no effect on fertility.

### Additional Information

RTECS: VZ2275000

---

## 12. ECOLOGICAL INFORMATION

### Toxicity

Toxicity to fish	LC50 - Carassius auratus (goldfish) - 178 mg/l - 72 h
Toxicity to daphnia and other aquatic invertebrates.	EC50 - Daphnia magna (Water flea) - 1,085 - 1,402 mg/l - 48 h
Toxicity to algae	IC50 - Desmodesmus subspicatus (green algae) - 158 mg/l - 96 h

### Persistence and degradability

no data available

### Bioaccumulative potential

no data available

### Mobility in soil

no data available

### PBT and vPvB assessment

no data available

### Other adverse effects

no data available

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## 13. DISPOSAL CONSIDERATIONS

### Product

Observe all federal, state, and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material.

### Contaminated packaging

Dispose of as unused product.

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## 14. TRANSPORT INFORMATION

### DOT (US)

Not dangerous goods

### IMDG

Not dangerous goods

### IATA

Not dangerous goods

---

## 15. REGULATORY INFORMATION

### OSHA Hazards

Teratogen, Reproductive hazard

### DSL Status

All components of this product are on the Canadian DSL list.

### SARA 302 Components

SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

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## Appendix C: MSDS for Sodium Borate

### SARA 313 Components

SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

### SARA 311/312 Hazards

Chronic Health Hazard

### Massachusetts Right To Know Components

	CAS-No.	Revision Date
Disodium tetraborate decahydrate	1303-96-4	1993-04-24

### Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Disodium tetraborate decahydrate	1303-96-4	1993-04-24

### New Jersey Right To Know Components

	CAS-No.	Revision Date
Disodium tetraborate decahydrate	1303-96-4	1993-04-24

### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

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## 16. OTHER INFORMATION

### Further information

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## **RACCOON (*Procyon lotor*)**

### **To begin necropsy:**

1. Position the animal on its back.
2. Cut the skin along the ventral midline from the chin to the tail.
3. Move the limbs out of the way by cutting through the muscles as well as the hip and shoulder joints and reflecting the limbs over to the side.



### **Removal of the head lymph nodes and tissues:**

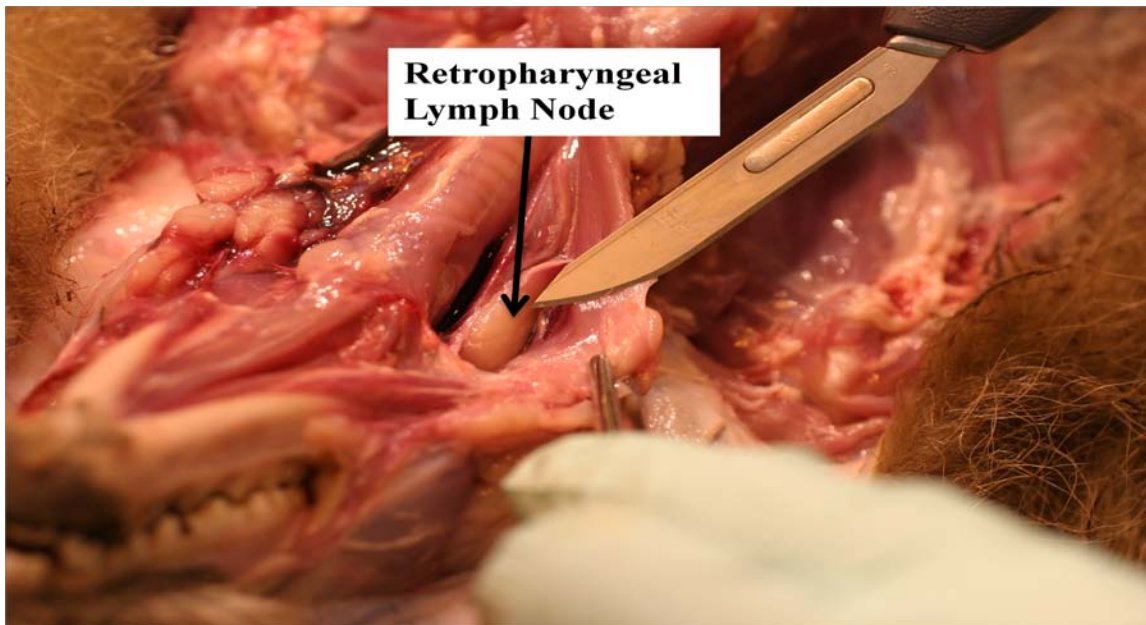
Extend the raccoon's neck and feel for the curvature of the mandible. The submandibular lymph nodes are just below the surface of the skin at the base of the mandible. The submandibular lymph nodes are associated with the salivary glands and can be easily confused. Be careful to collect lymph node and not cut too deep when cutting through the skin.





## Appendix D: Raccoon Necropsy

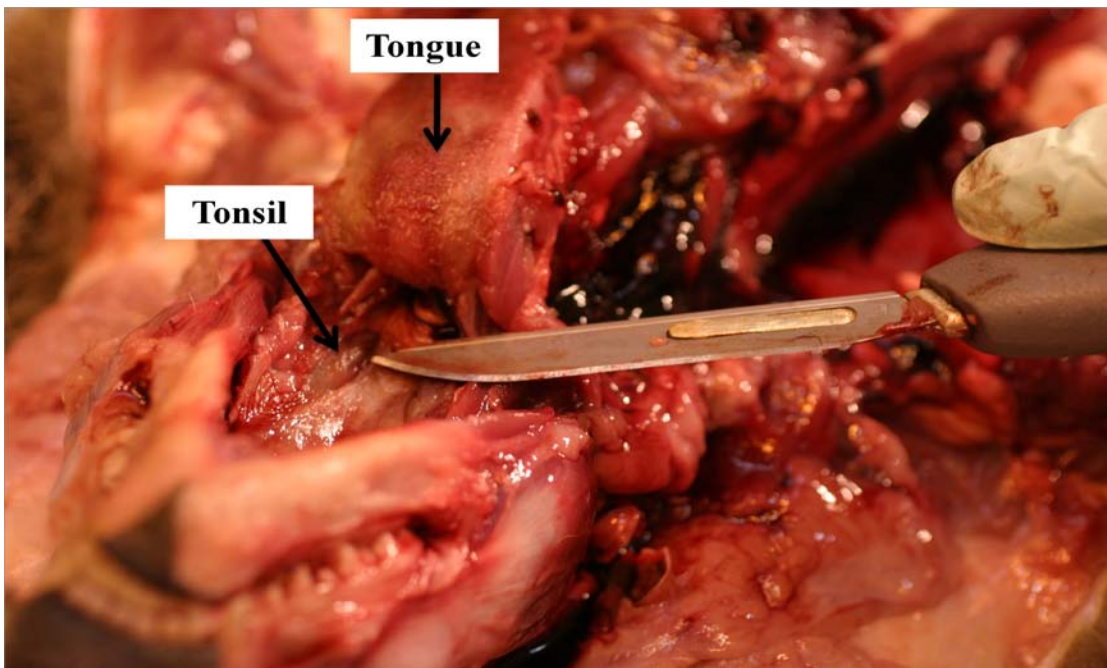
After removing the submandibular lymph nodes, cut from the curvature of the mandible along the side of the trachea, carefully separating the muscles to avoid damaging the retropharyngeal lymph node. Remove both medial retropharyngeal lymph nodes.



It is not necessary to collect the parotid lymph nodes from raccoons because they are small and difficult to find in animals smaller than cervids.

### Removal of the tonsils:

To remove the tonsils, free the tongue by cutting between the sides and tip of the tongue and the mandible. Retract the tongue towards the tail. The tonsils can be seen on the soft palate of the mouth on either side. Carefully extract both tonsils.

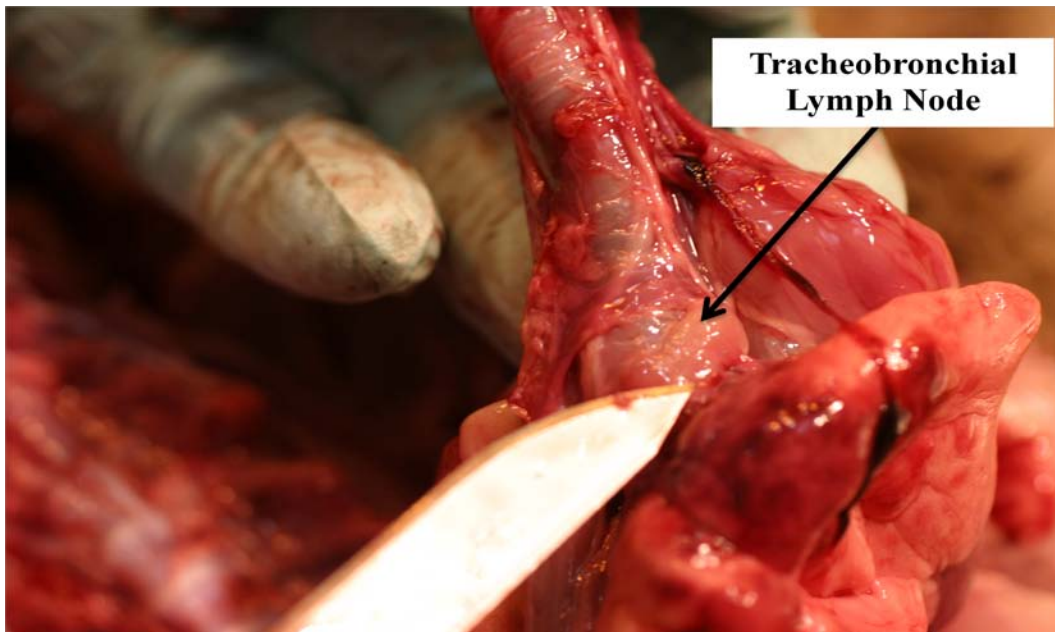


## Appendix D: Raccoon Necropsy

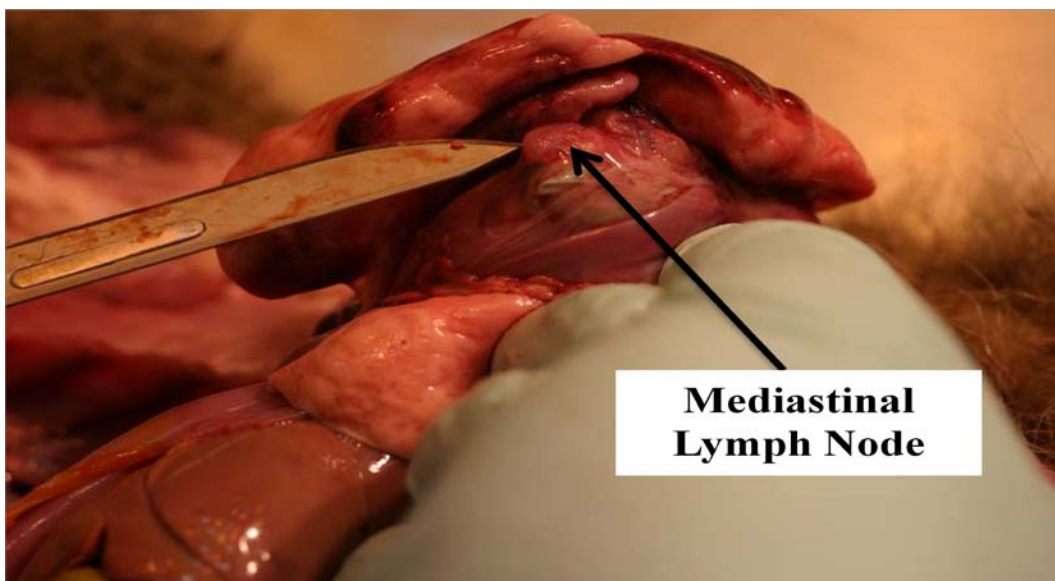
### Removal of the thoracic lymph nodes and tissues:

Expose the chest cavity by cutting the ribs along the vertebrae and rotate the rib cage up and above the carcass. Alternatively, the rib cage can be cut along the sternum and along the back and removed. Visually examine and palpate the lungs for firmness, lumps, or lesions. Also, look for lesions or abnormalities on the wall of the chest or elsewhere in the chest cavity. If in doubt, submit the tissue to the laboratory for a conclusive interpretation.

Identify where the trachea branches into the right and left bronchii. The tracheobronchial lymph nodes are located where the main stem bronchii branch off of the trachea. The aortic arch surrounds the left tracheobronchial lymph node.



The mediastinal lymph node is located on the front side of the junction of the bronchii and the heart. When holding the heart, flip it over and push your finger between the two divisions—the mediastinal lymph node should then pop out.

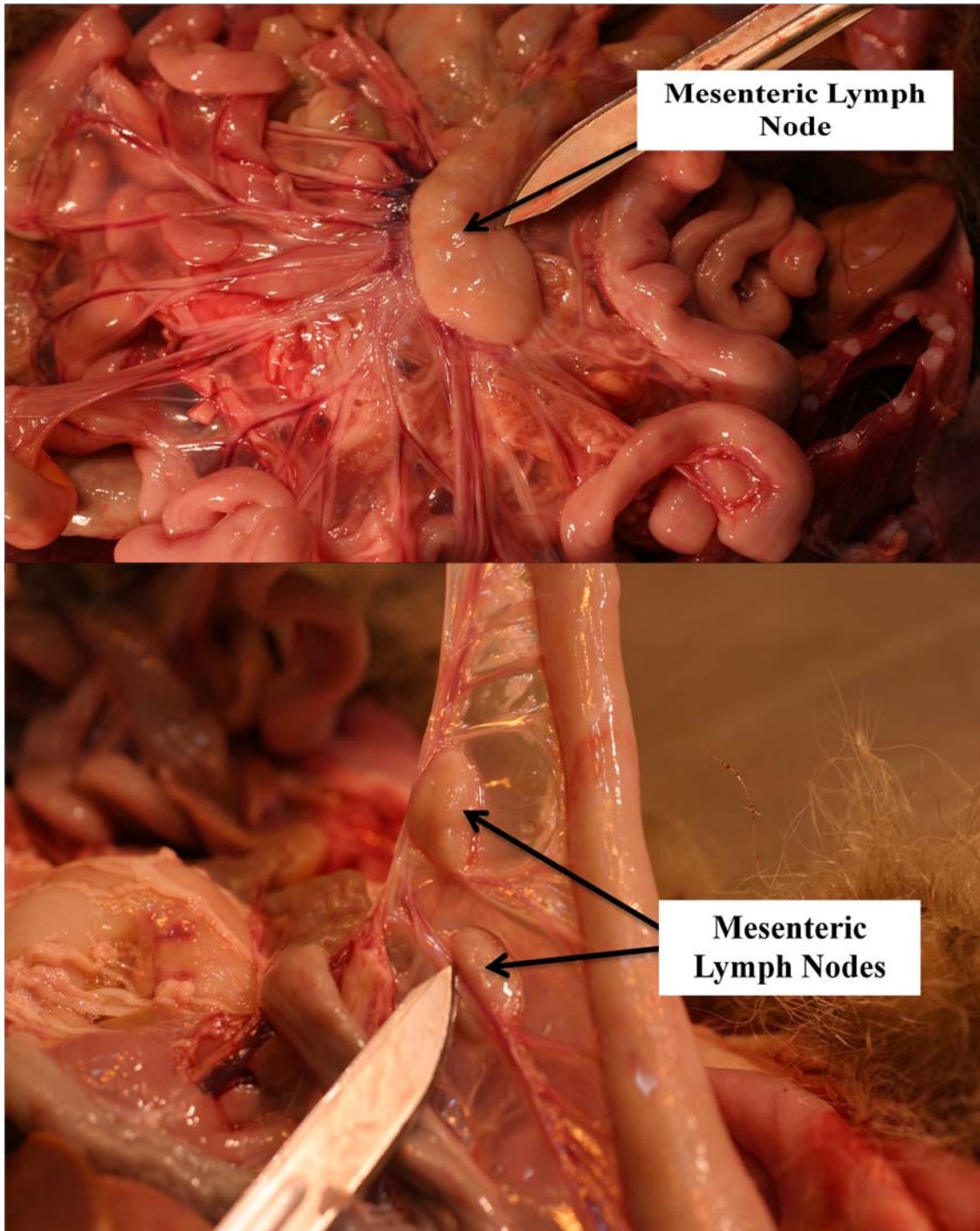




## Appendix D: Raccoon Necropsy

### Removal of abdominal cavity lymph nodes:

The mesenteric lymph nodes are the only lymph nodes from the abdominal cavity to routinely submit to the laboratory. Expose the abdominal contents by removing the flap of muscle that covers the abdominal cavity from the rib cage to the pelvis. The mesenteric lymph nodes are located within the thin, opaque mesentery that suspends the bowel. The mesenteric lymph nodes most readily apparent are in the mesentery between the loops of small intestine where the blood vessels converge. There are also several smaller mesenteric lymph nodes between the large intestine and kidneys.



## DEER (Family *Cervidae*)

### To begin necropsying:

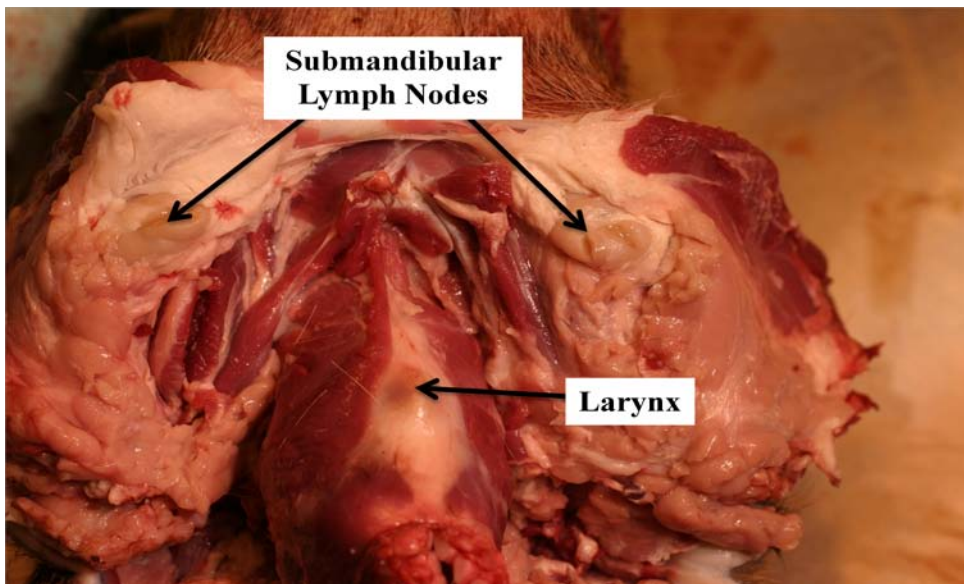
1. Place the deer on its side.
2. Cut the skin along the ventral midline from the chin to the tail.
3. Move the limbs out of the way by cutting through the muscles as well as the hip and shoulder joints and reflecting the limbs over to the side.

### Removal of the head lymph nodes and tissues:

Feel for the curvature of the mandible and the larynx and cut between the two.



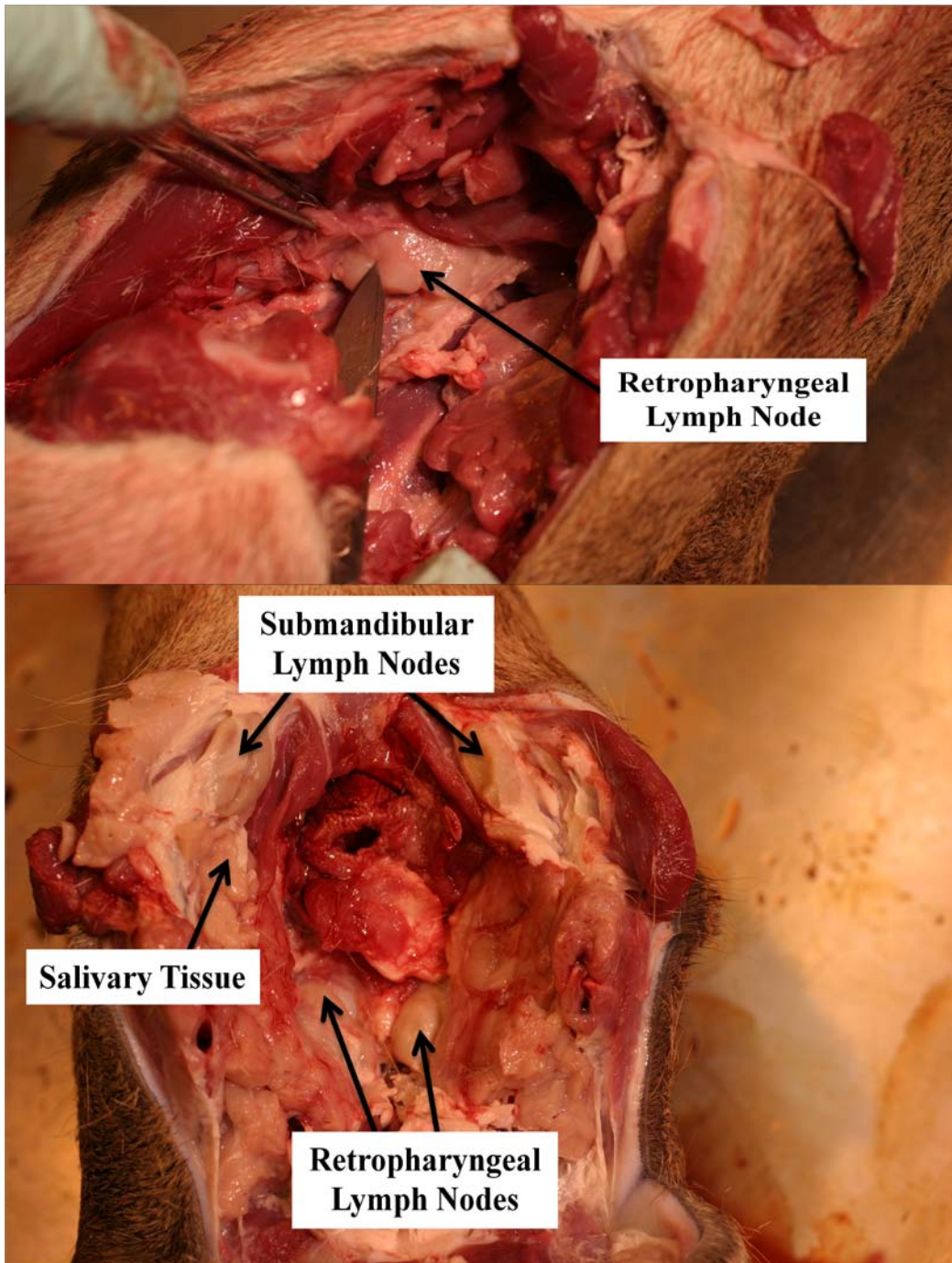
The submandibular lymph nodes are associated with the mandibular salivary glands located just under the skin near the curvature of the mandible.





## Appendix E: Deer and Other Cervid Necropsy

After removing the submandibular lymph nodes, cut through the trachea in front of the larynx and look for the lymph node in front of the salivary glands. Remove both medial retropharyngeal lymph nodes.



Salivary tissue surrounds both the retropharyngeal and submandibular lymph nodes and can be easily confused with lymph nodes. Be careful to identify and collect only the lymph nodes and not the salivary gland. Lymph nodes are solid white-gray in color and are not lobulated; adjacent salivary glands are lobulated.

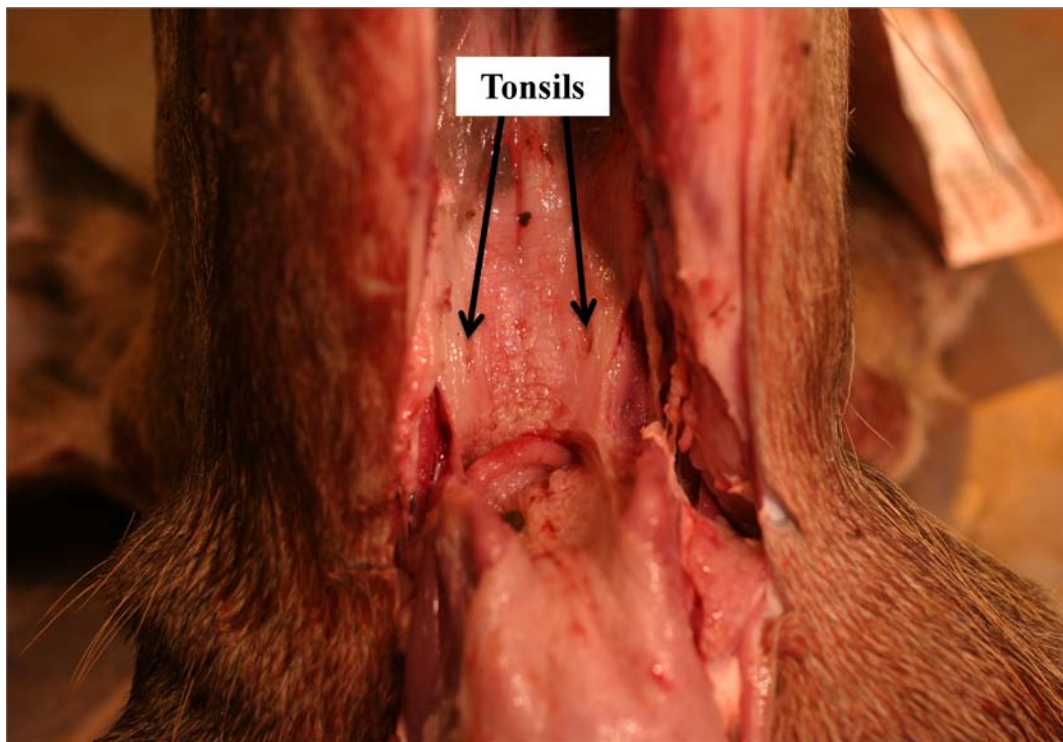
## Appendix E: Deer and Other Cervid Necropsy

The parotid lymph nodes are located within the parotid salivary gland between the ear and the eye, just in front of and below the external ear canal. Locate and extract both parotid lymph nodes on either side of the head. Feel for the slight indentation of the temple to begin the cut.



### Removal of the tonsils:

To remove the tonsils, open the mandible and pull back the tongue. The tonsils can be seen on the roof the mouth on either side. Carefully extract both tonsils.



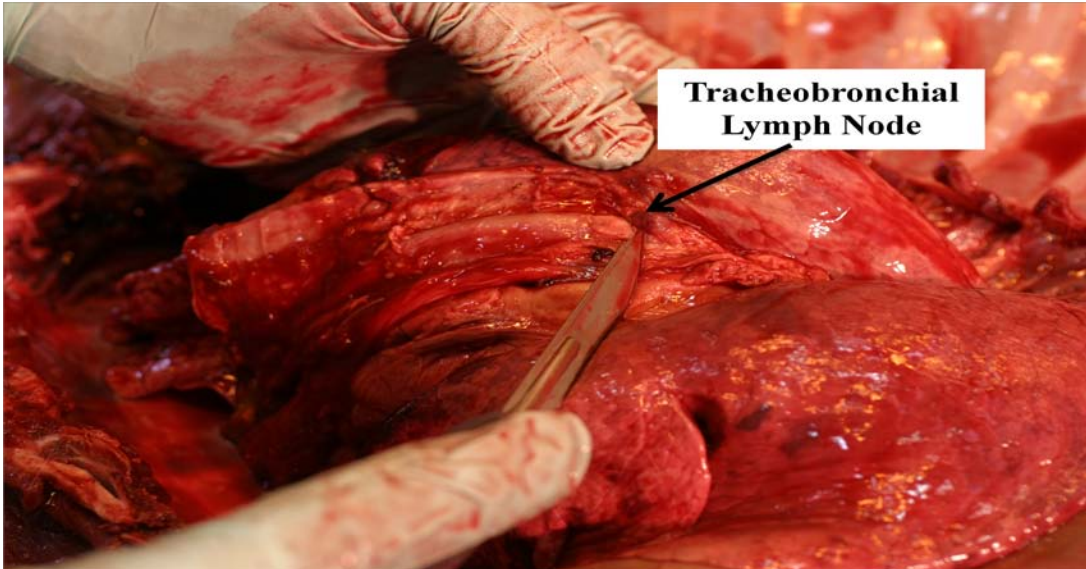


## Appendix E: Deer and Other Cervid Necropsy

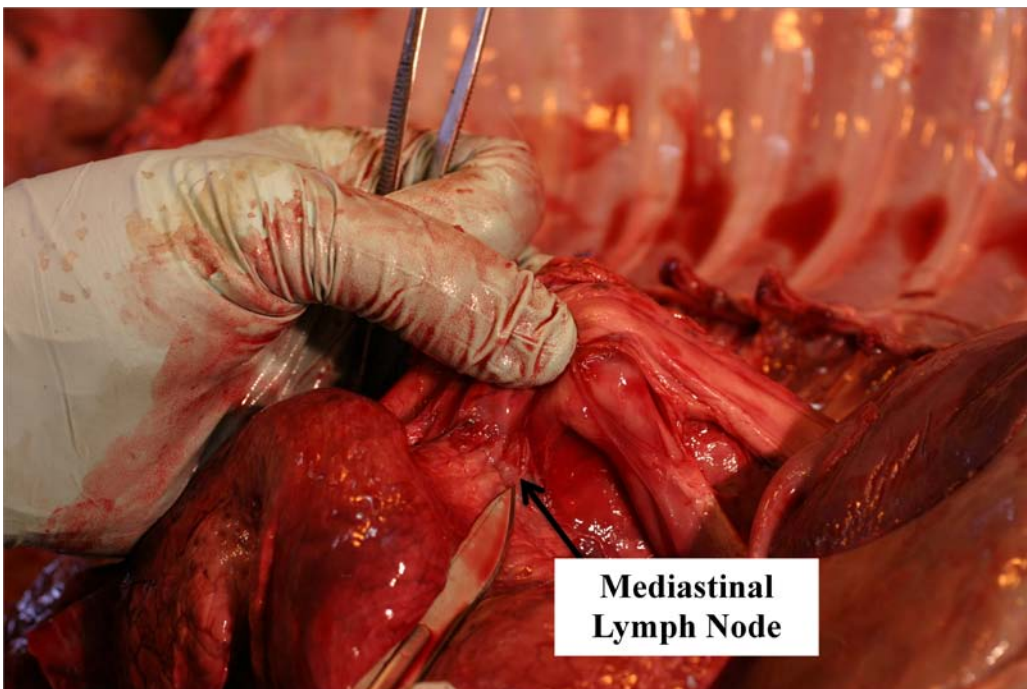
### Removal of the thoracic lymph nodes and tissues:

Expose the chest cavity by cutting the ribs along the sternum and backbone. Visually examine and palpate the lungs for firmness, lumps, or lesions. Also, look for lesions on the wall of the chest or elsewhere in the chest cavity for abnormalities. If in doubt, submit the tissue and let the laboratory decide.

Identify where the trachea branches into the right and left bronchii. The tracheobronchial lymph nodes are located on the left and right sides where the bronchii branch. The aortic arch surrounds the left tracheobronchial lymph node.



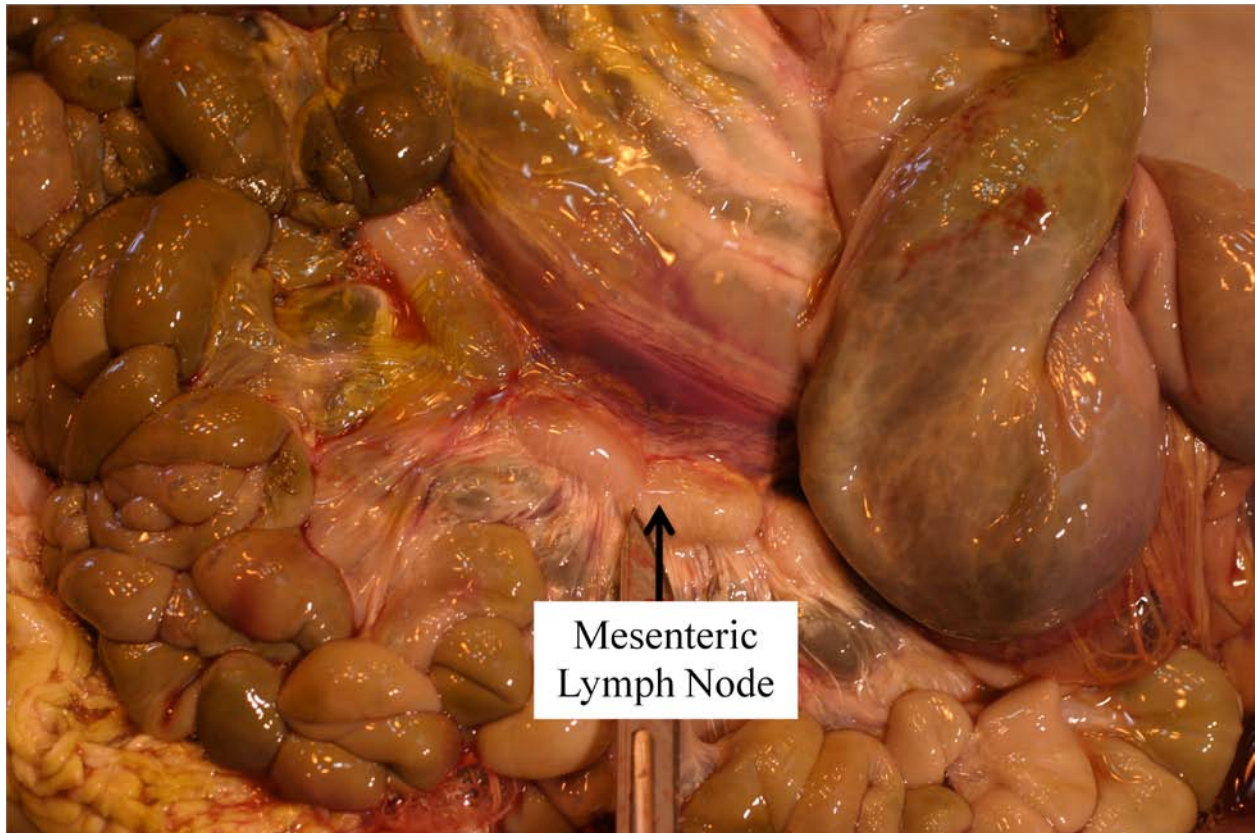
The mediastinal lymph node is located on the ventral side of the junction of the bronchii and the heart. When holding the heart, flip it over and push your finger between the two divisions—the mediastinal lymph node should then pop out.



## Appendix E: Deer and Other Cervid Necropsy

### Removal of the abdominal cavity lymph nodes:

Expose the abdominal contents by removing the flap of muscle that covers the abdominal cavity. The mesenteric lymph nodes are the only lymph nodes from the abdominal cavity to routinely submit to the laboratory. The mesenteric lymph nodes are located between the small intestine and the cecum in the mesentery between the loops of the small intestine. The mesenteric lymph nodes are usually elongated in an arc or crescent, and generally appear as a series of lymph nodes. Use the cecum as a landmark for the termination of the small intestine and work backwards.





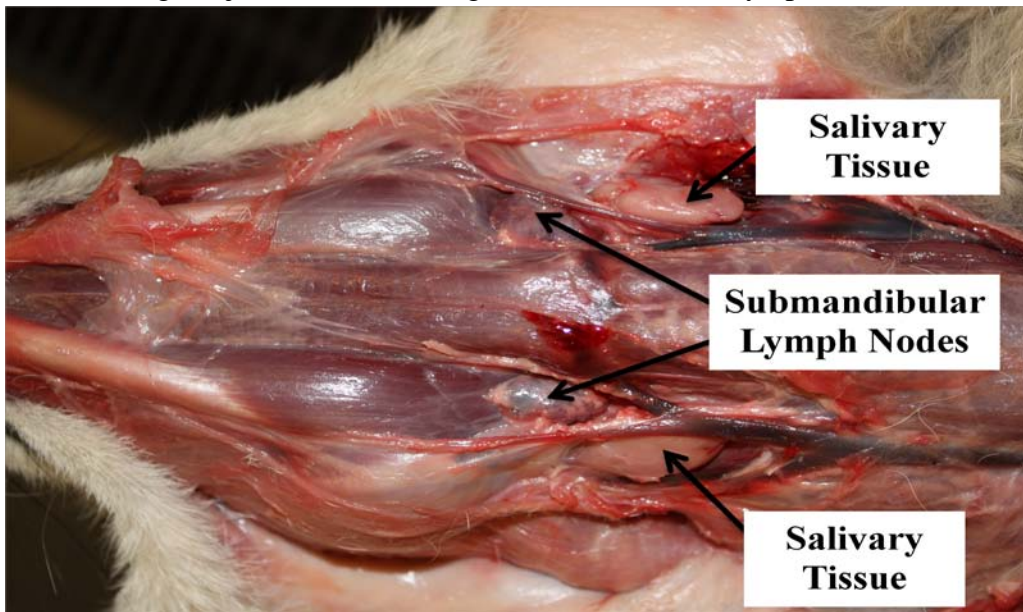
## Coyote (*Canis latrans*)

### To begin necropsy:

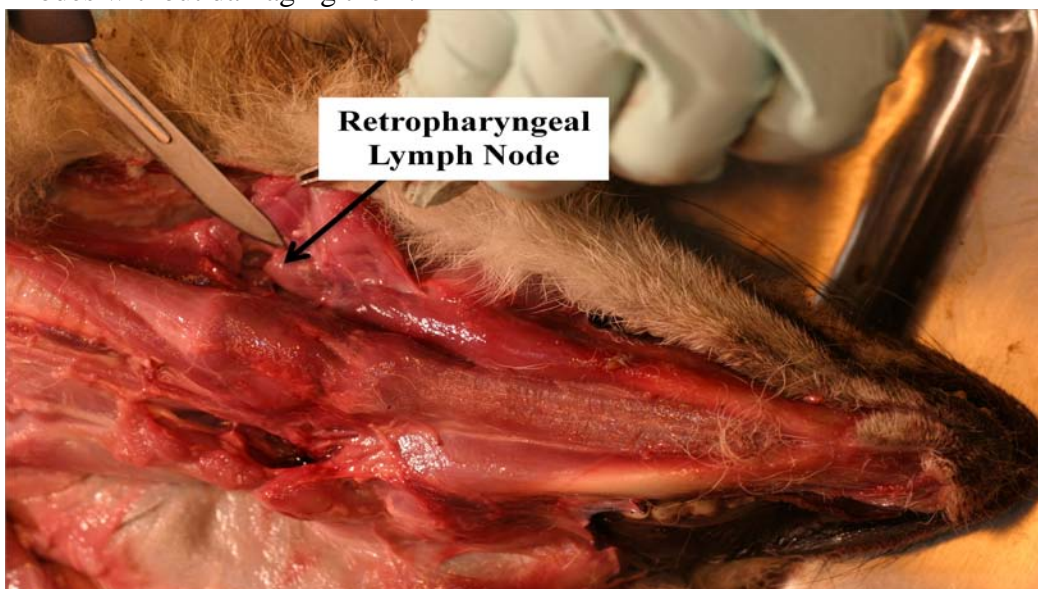
1. Place the animal on its back and cut the skin along the ventral midline from the chin to the tail.
2. Move the limbs out of the way by cutting through the muscles as well as the hip and shoulder joints and reflecting the limbs over to the side.

### Removal of the head lymph nodes and tissues:

Extend the coyote's neck and feel for the curvature of the mandible. The submandibular lymph nodes are just below the surface of the skin at the base of the mandible. Be careful not to cut too deep when skinning the jaw to avoid cutting the submandibular lymph nodes.



After removing the submandibular lymph nodes, cut from the curvature of the mandible along the side of the trachea, carefully separating the muscles to expose the medial retropharyngeal lymph nodes without damaging them.

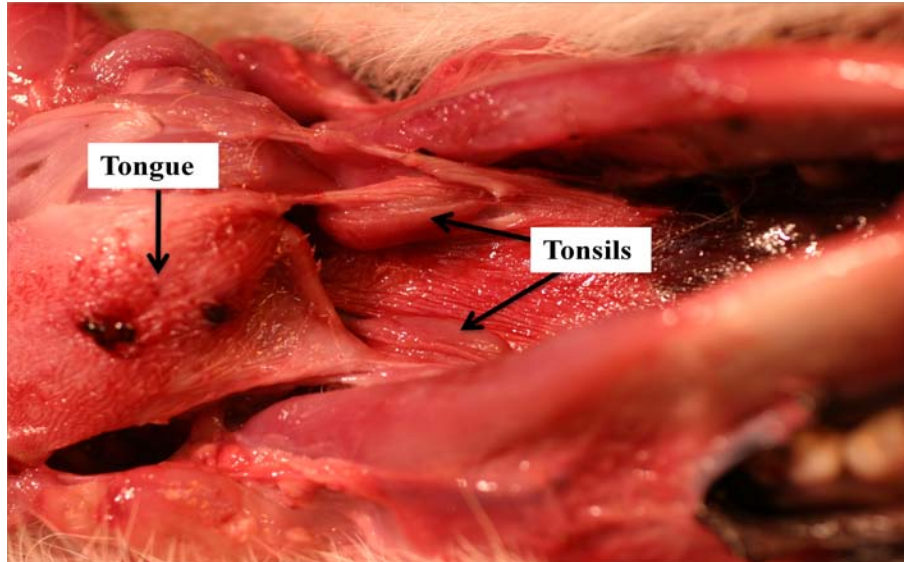


## Appendix F: Coyote Necropsy

It is not necessary to collect the parotid lymph nodes from coyotes because they are small and difficult to find in animals smaller than cervids.

### Removal of the tonsils:

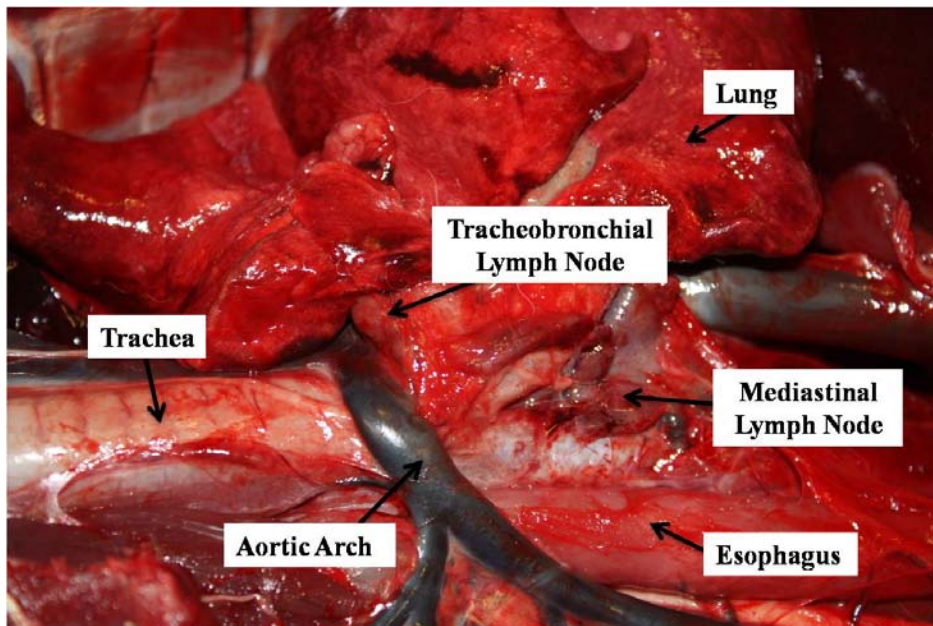
To remove the tonsils, open the mandible and pull back the tongue. The tonsils can be seen on the roof the mouth on either side of the soft palate. Carefully extract both tonsils.



### Removal of the thoracic lymph nodes and tissues:

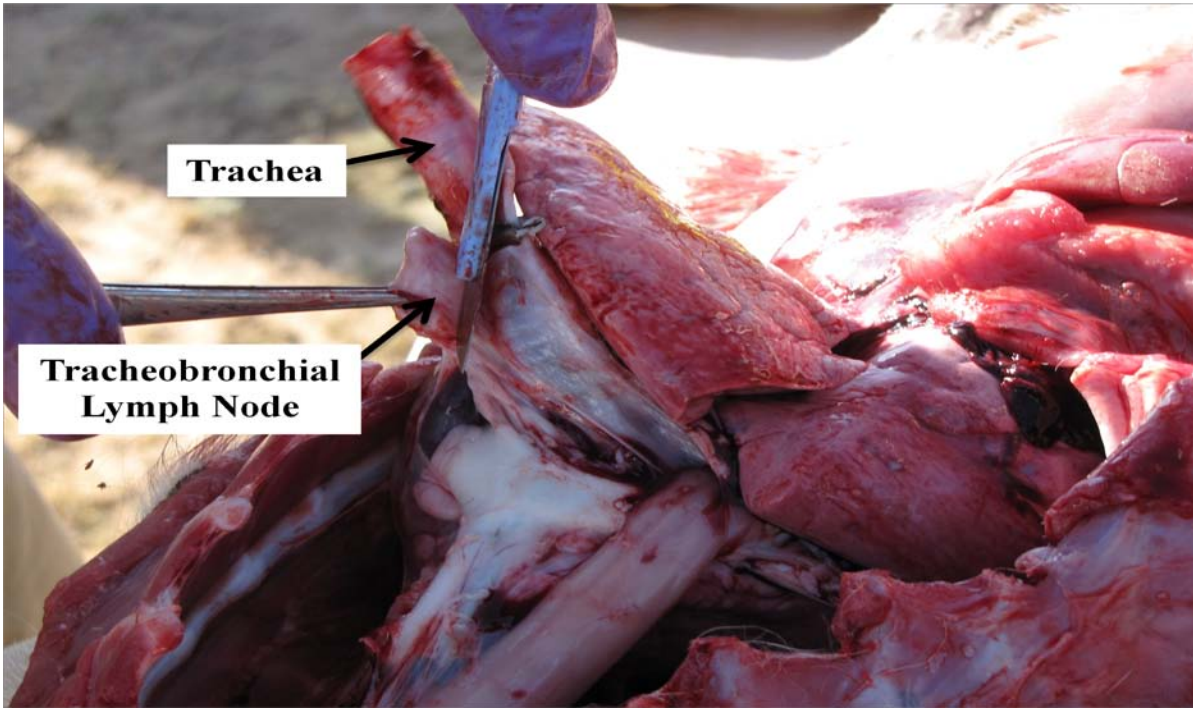
Expose the chest cavity by cutting the ribs along the vertebrae. Visually examine and palpate the lungs for firmness, lumps, or lesions. Also, look for lesions on the wall of the chest or elsewhere in the chest cavity for abnormalities. If in doubt, submit the tissue and let the lab make the decision.

Identify where the trachea branches into the right and left bronchii. The tracheobronchial lymph nodes are located where the bronchii branch. The aortic arch surrounds the left tracheobronchial lymph node.

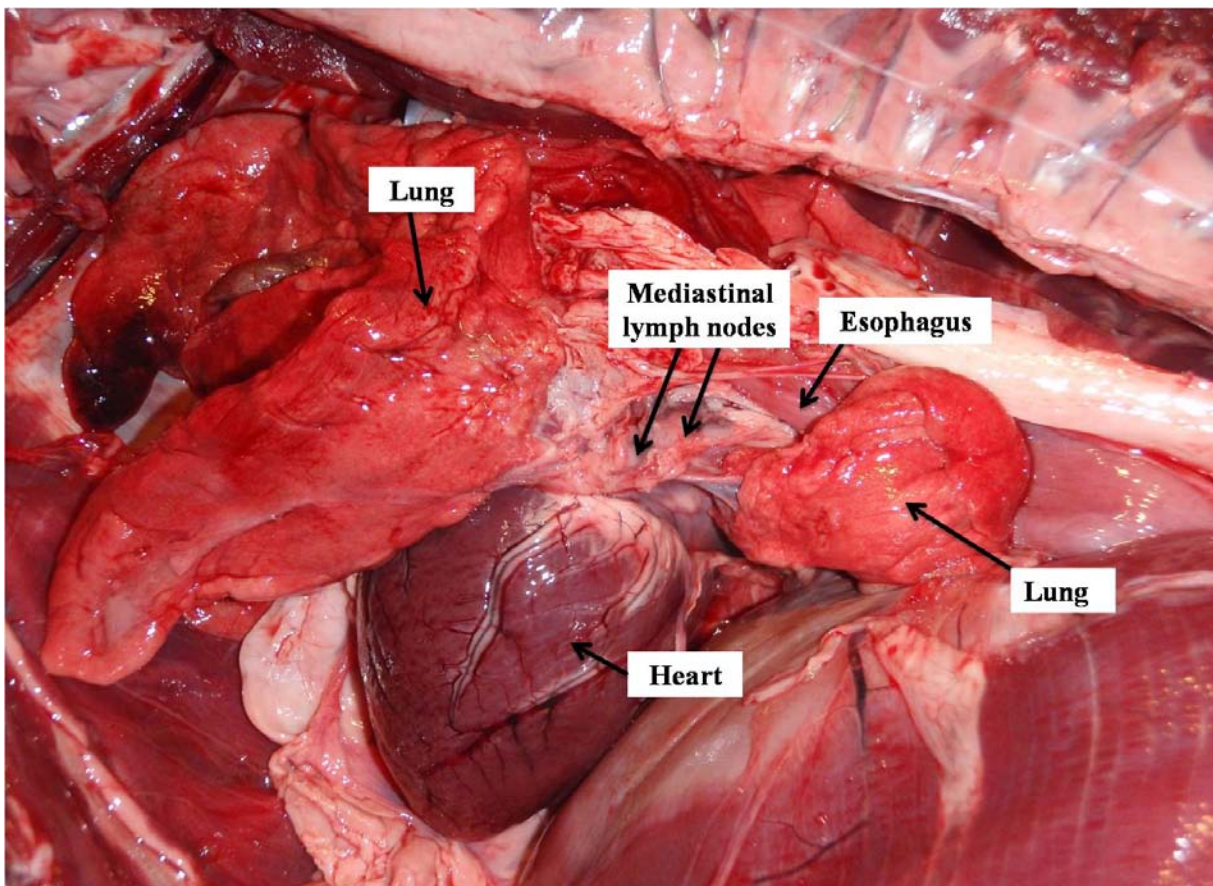




## Appendix F: Coyote Necropsy



The mediastinal lymph node is located on the ventral side of the junction of the bronchii with the heart. When holding the heart, flip it over and push your finger between the two divisions—the mediastinal lymph node should pop out.

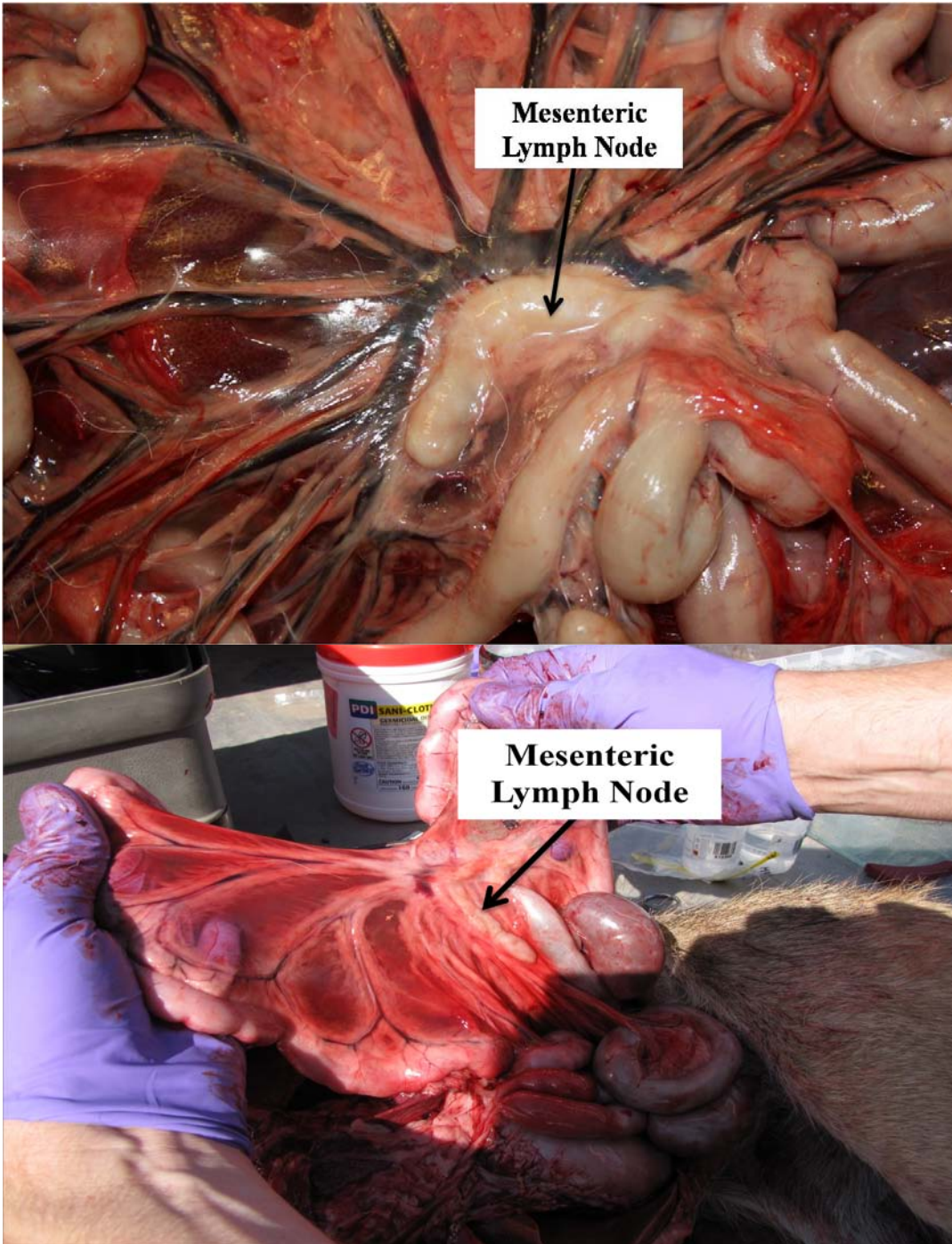




## Appendix F: Coyote Necropsy

### Removal of the abdominal cavity lymph nodes:

Expose the abdominal contents by removing the flap of muscle that covers the abdominal cavity. The mesenteric lymph nodes are the only lymph nodes to routinely submit from the abdominal cavity. There are multiple mesenteric lymph nodes in the middle of the mesentery of the small intestine. They are most readily apparent in the mesentery between the loops of small intestine where the blood vessels converge. There are several smaller mesenteric lymph nodes in the mesentery between the large intestine and the kidneys.



## Opossum (*Didelphis virginiana*)

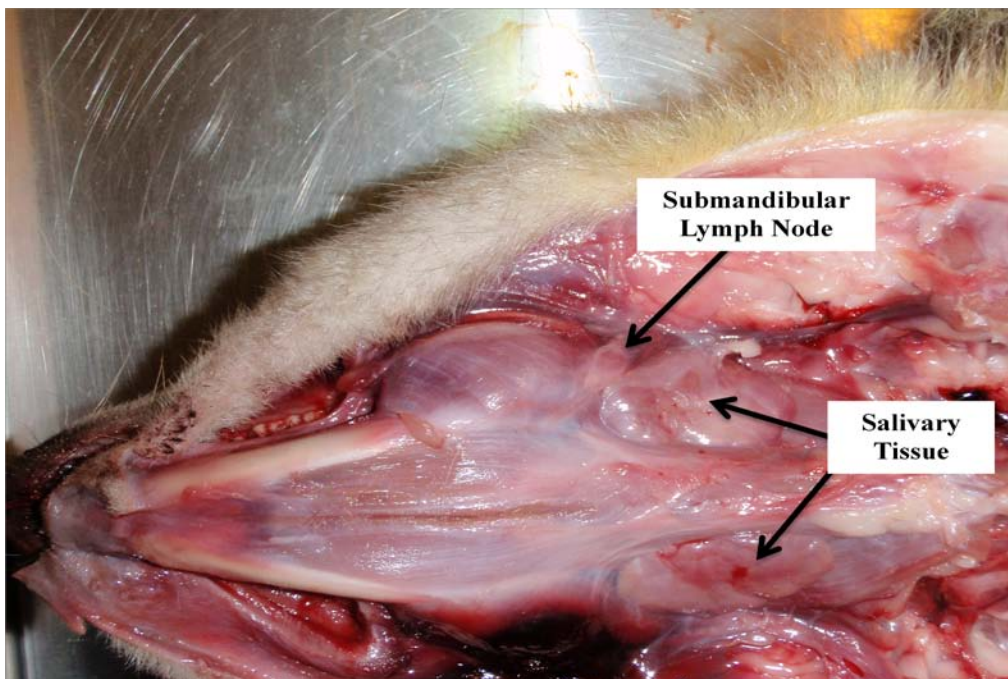
### To begin necropsy:

1. Place the animal on its back and cut the skin along the ventral midline from chin to tail.
2. Move the limbs out of the way by cutting through the muscles, as well as the hip and shoulder joints, and reflecting the limbs over to the side.



### Removal of the head lymph nodes and tissues:

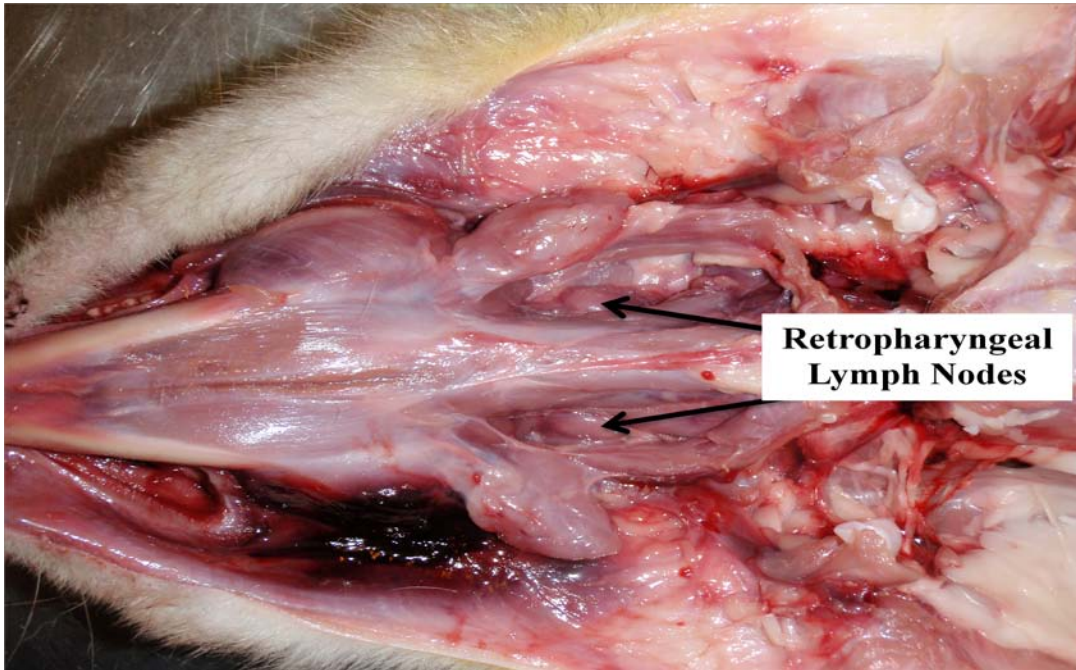
Extend the opossum's neck and feel for the curvature of the mandible. The submandibular lymph nodes are just below the surface of the skin at the base of the mandible. Be careful not to cut too deep when cutting through the skin.





## Appendix G: Opossum Necropsy

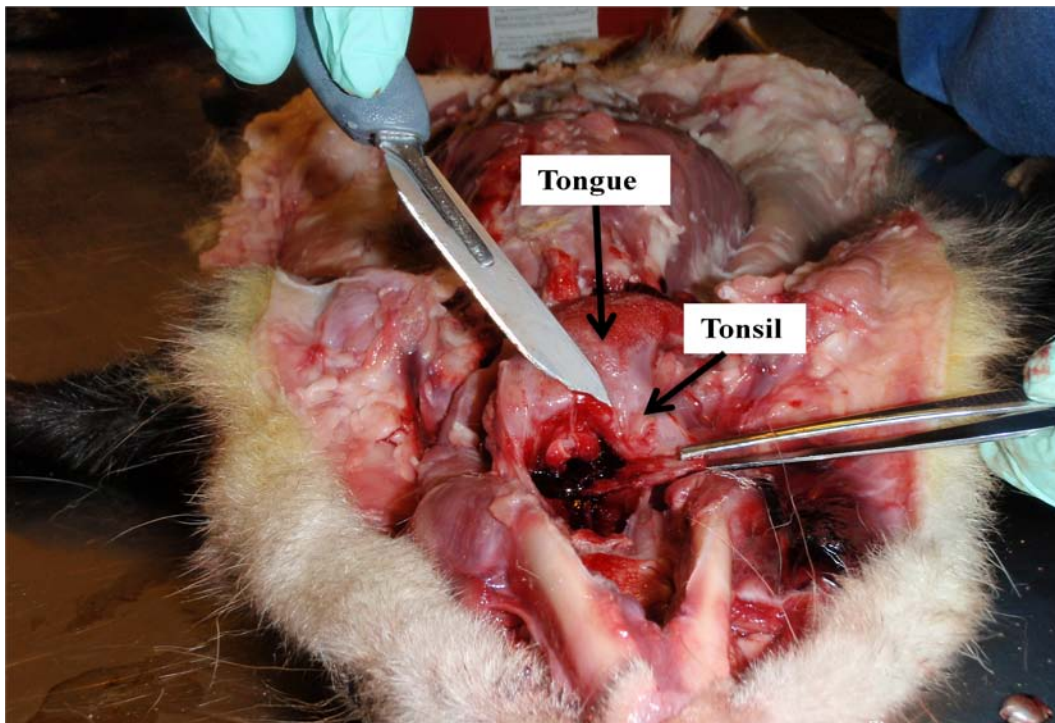
After removing the submandibular lymph nodes, cut from the curvature of the mandible along the side of the trachea; carefully separating the muscles to avoid damaging the medial retropharyngeal lymph nodes. Remove both medial retropharyngeal lymph nodes.



It is not necessary to collect the parotid lymph nodes from opossums because they are small and difficult to find in animals smaller than cervids.

### Removal of the tonsils:

To remove the tonsils, loosen the tongue by cutting along the sides and pull the tongue back. The tonsils can be seen where the tongue connects to the soft palate. Carefully extract both tonsils.

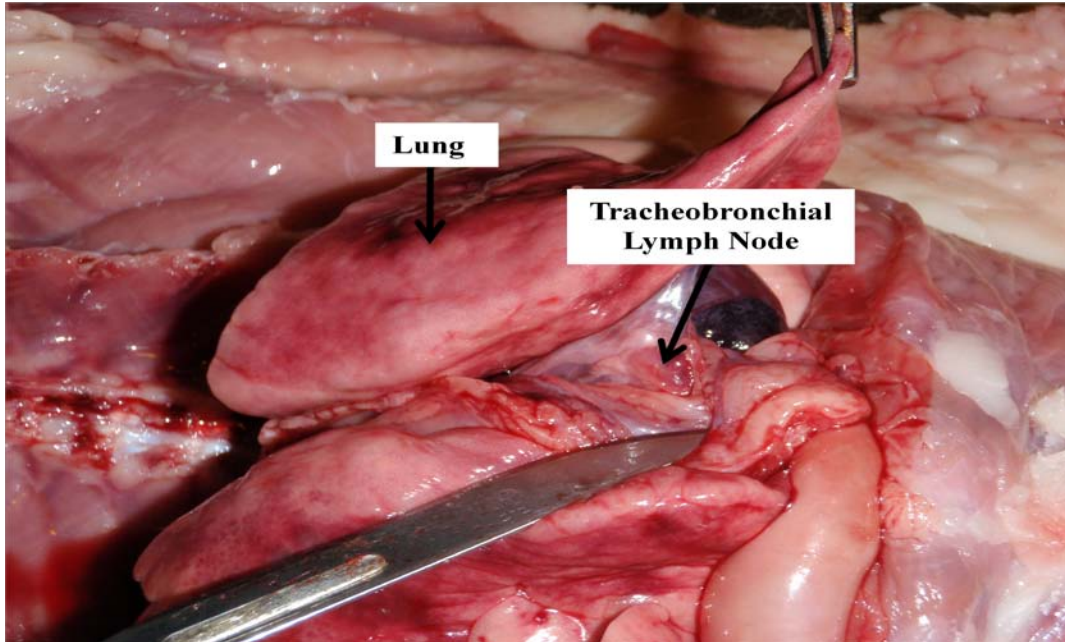


## Appendix G: Opossum Necropsy

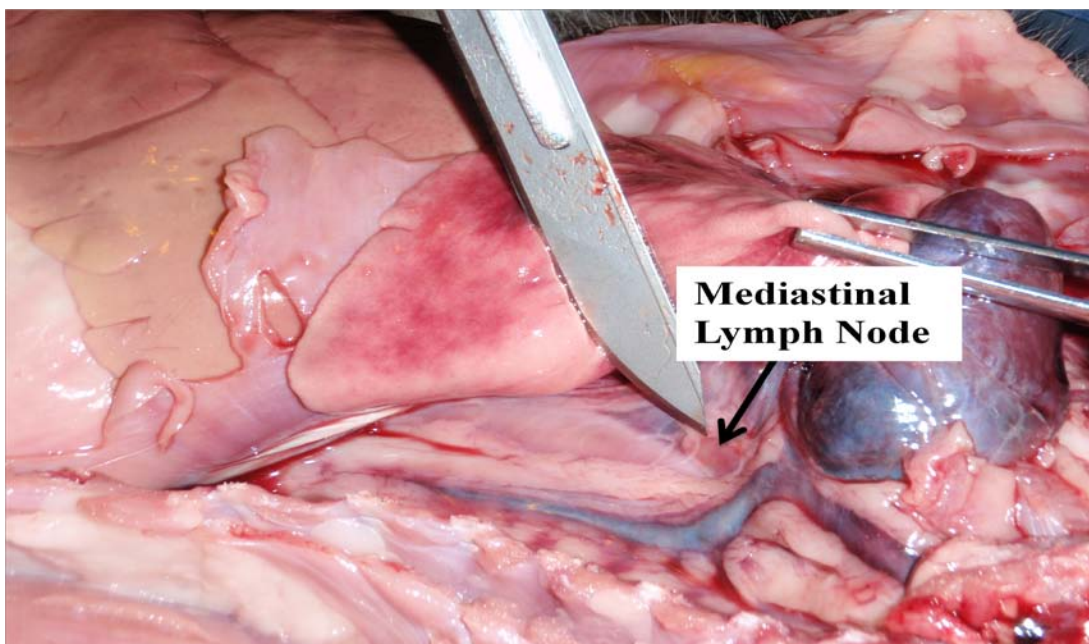
### Removal of the thoracic lymph nodes and tissues:

Expose the chest cavity by cutting the ribs along the sternum. Visually examine and palpate the lungs for firmness, lumps, or lesions. Collect samples for culture and histopathology, and look for lesions on the wall of the chest or elsewhere in the chest cavity for abnormalities. If in doubt, submit the tissue and let the laboratory decide.

Identify the area where the trachea branches into the right and left bronchii. The tracheobronchial lymph nodes are located where the bronchii branch. The aortic arch surrounds the left tracheobronchial lymph node.



The mediastinal lymph node is located on the ventral side of the junction of the bronchii and the heart. When holding the heart, flip it over and push your finger between the two divisions—the mediastinal lymph node should then pop out.

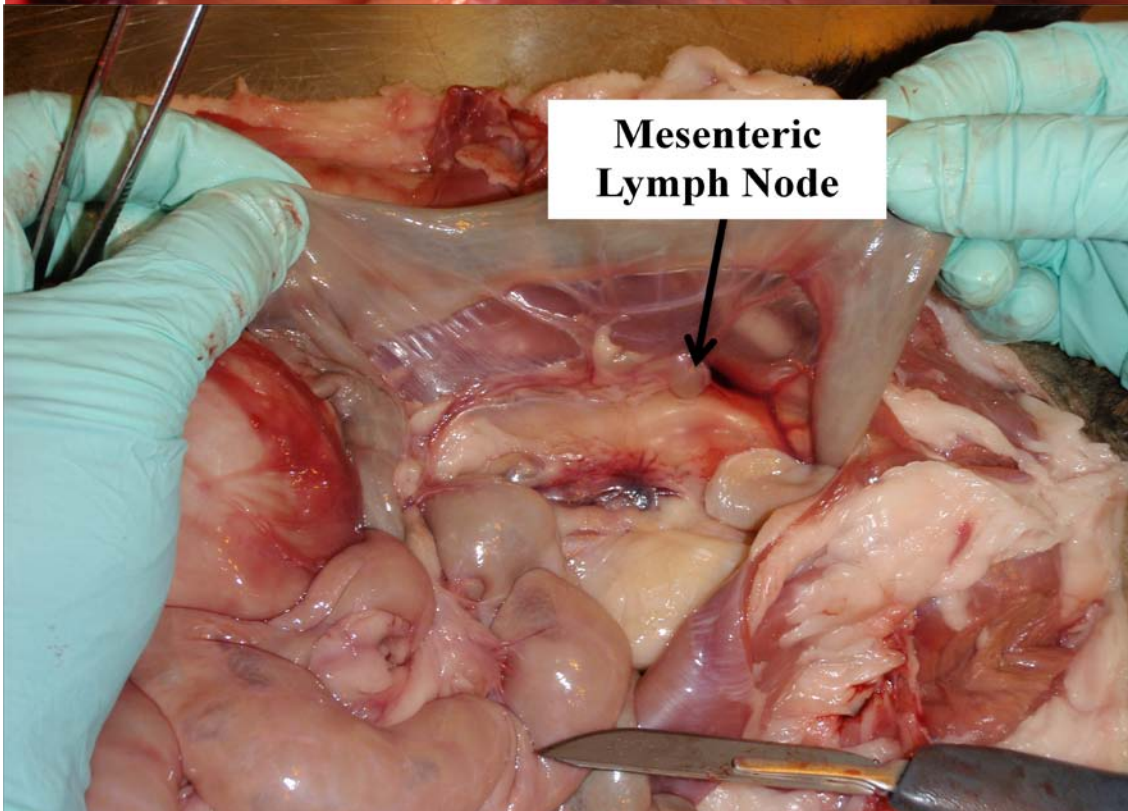
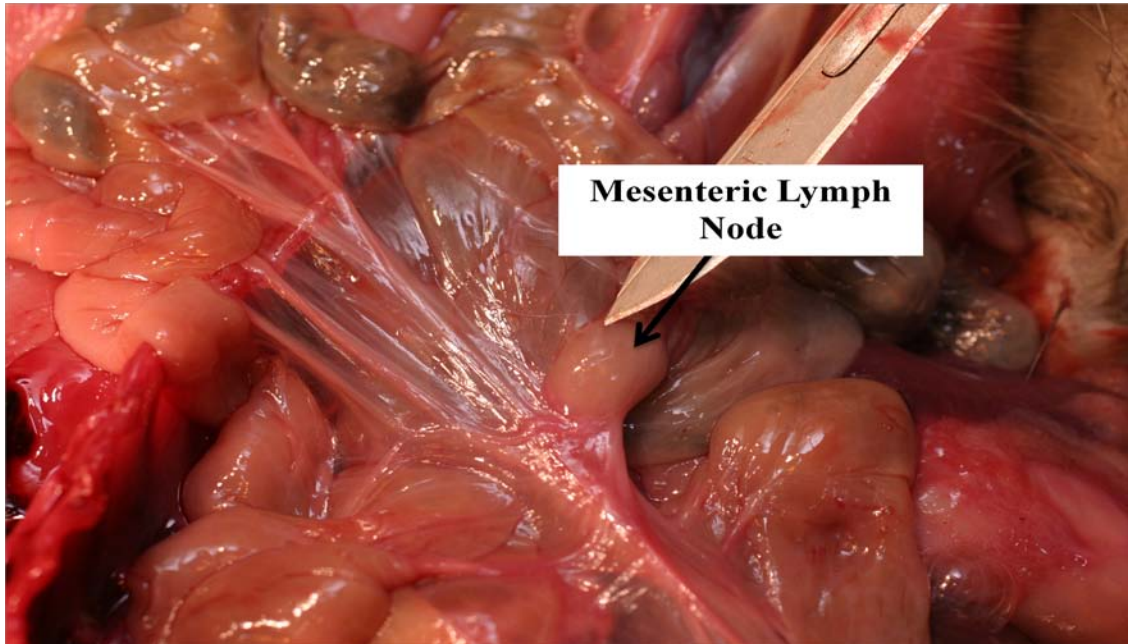




## Appendix G: Opossum Necropsy

### Removal of the abdominal cavity lymph nodes:

Expose the abdominal contents by removing the flap of muscle that covers the abdominal cavity from the rib cage to the pelvis. The mesenteric lymph nodes are the only lymph nodes from the abdominal cavity to submit to the laboratory. They are located within the thin, opaque mesentery that suspends the bowel, and are most readily apparent in the mesentery between the loops of small intestine where the blood vessels converge. There are also several mesenteric lymph nodes between the large intestine and kidneys.





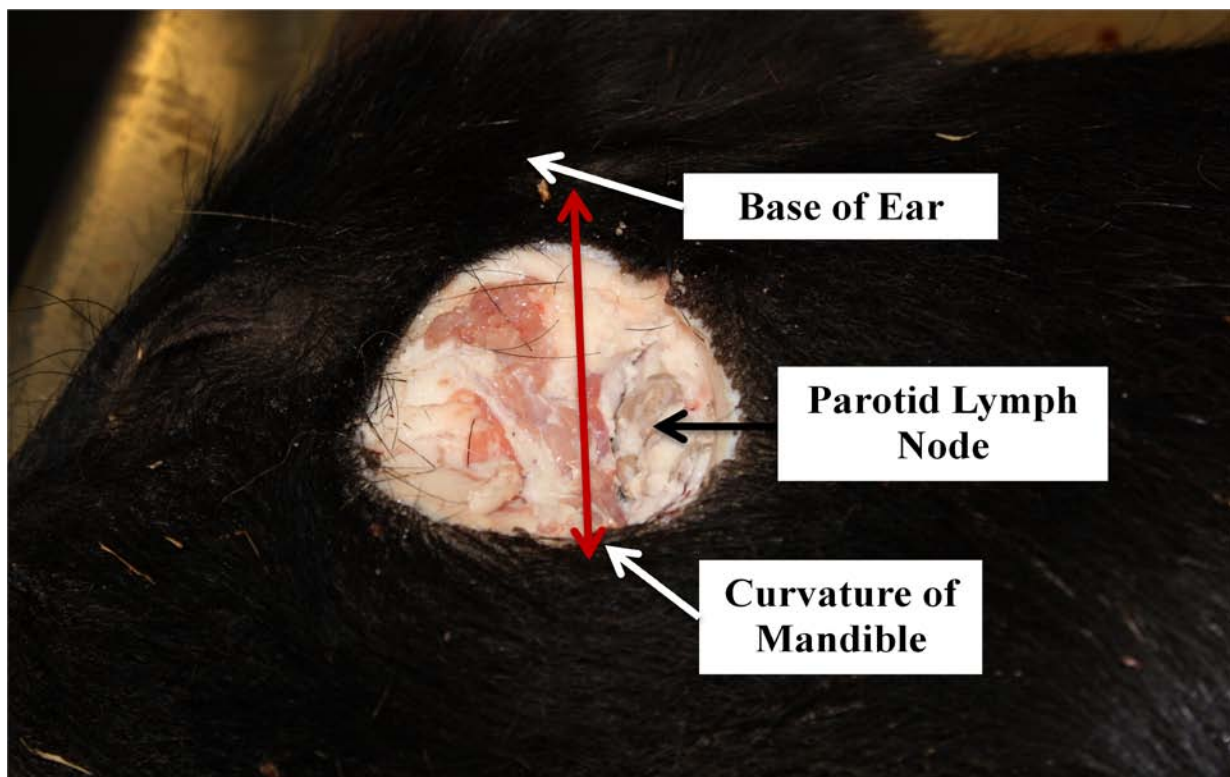
## **Feral Swine (*Sus scrofa*)**

### **To begin necropsy:**

1. Place the animal on its side and cut the skin along the ventral midline from chin to tail.
2. Move the limbs out of the way by cutting through the muscles as well as the hip and shoulder joints and reflecting the limbs over to the side.

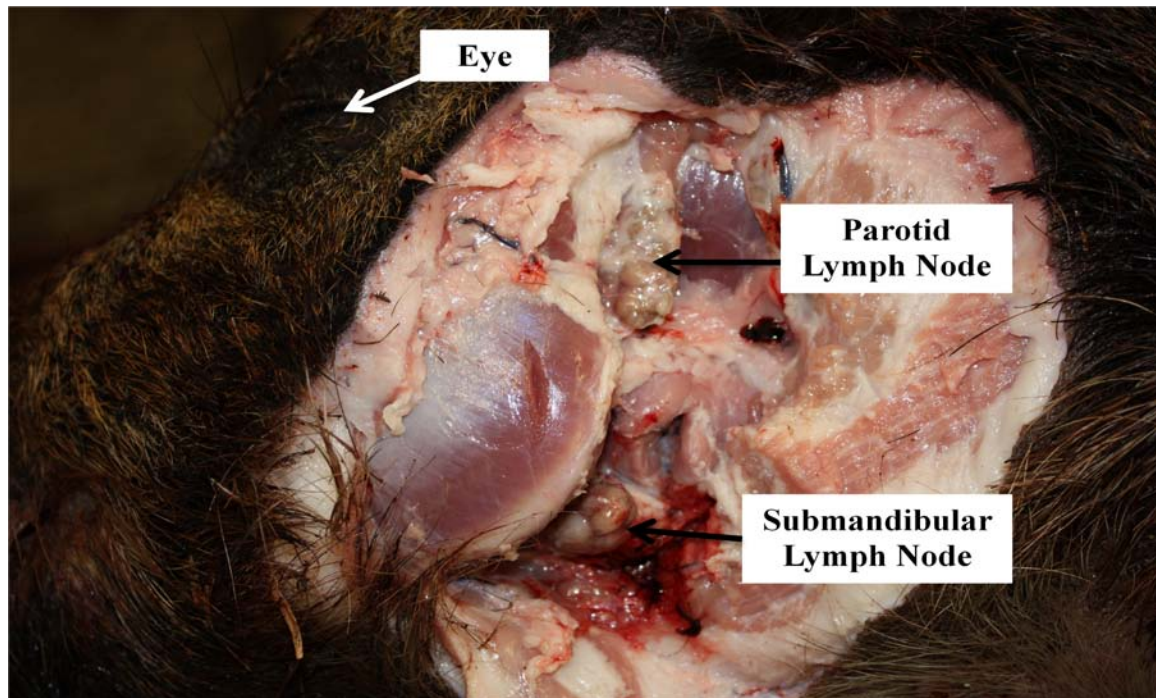
### **Removal of the head lymph nodes and tissues:**

Feral swine have more fat than other wildlife species; therefore, the lymph nodes may be deeper than in other species. Feel for the curvature of the mandible and find the base of the ear canal. The parotid lymph node is located on the caudal side of the line between the curvature of the mandible and the base of the ear canal.

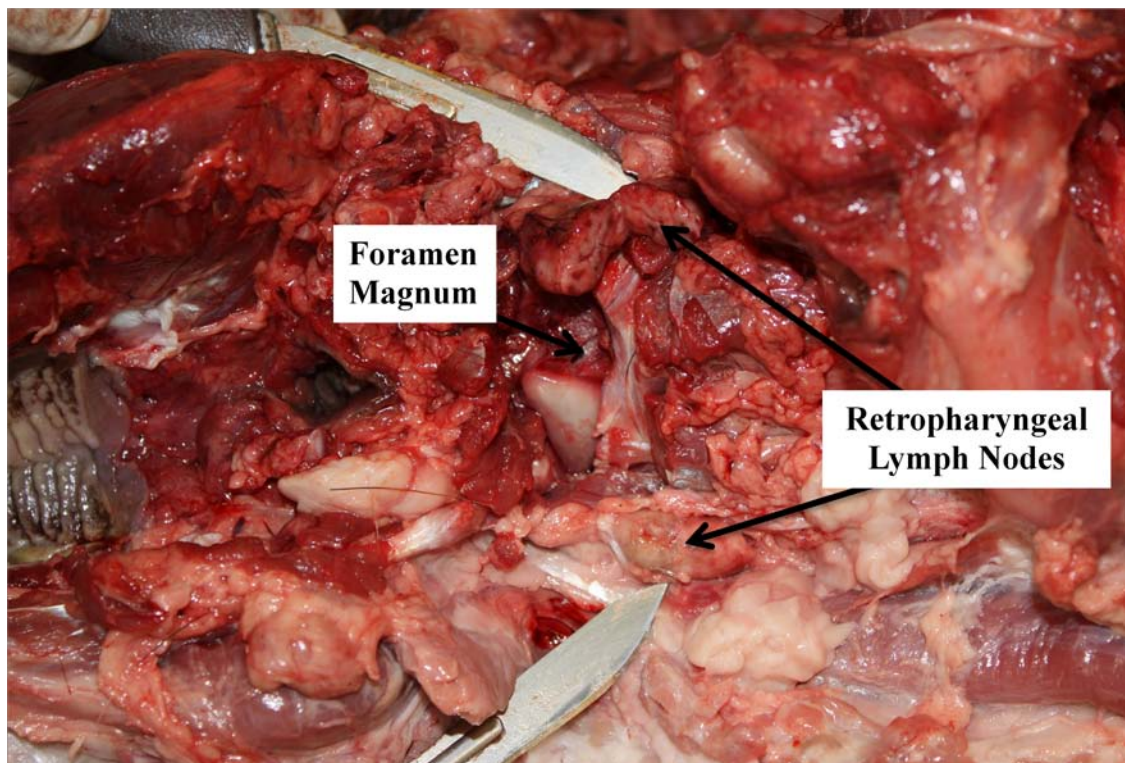


The submandibular lymph nodes are located on the interior side of the curvature of the mandible. They are multilobular and appear very different than other species. Lymph nodes in feral swine are often multilobulated and multiple lymph nodes appear rather than singly.

## Appendix H: Feral Swine Necropsy



After removing the submandibular lymph nodes, cut towards the foramen magnum where the neck hinges. The medial retropharyngeal lymph nodes are more caudal than in other species and are directly behind the paramastoid process (bony structures in the skull). Remove both medial retropharyngeal lymph nodes.

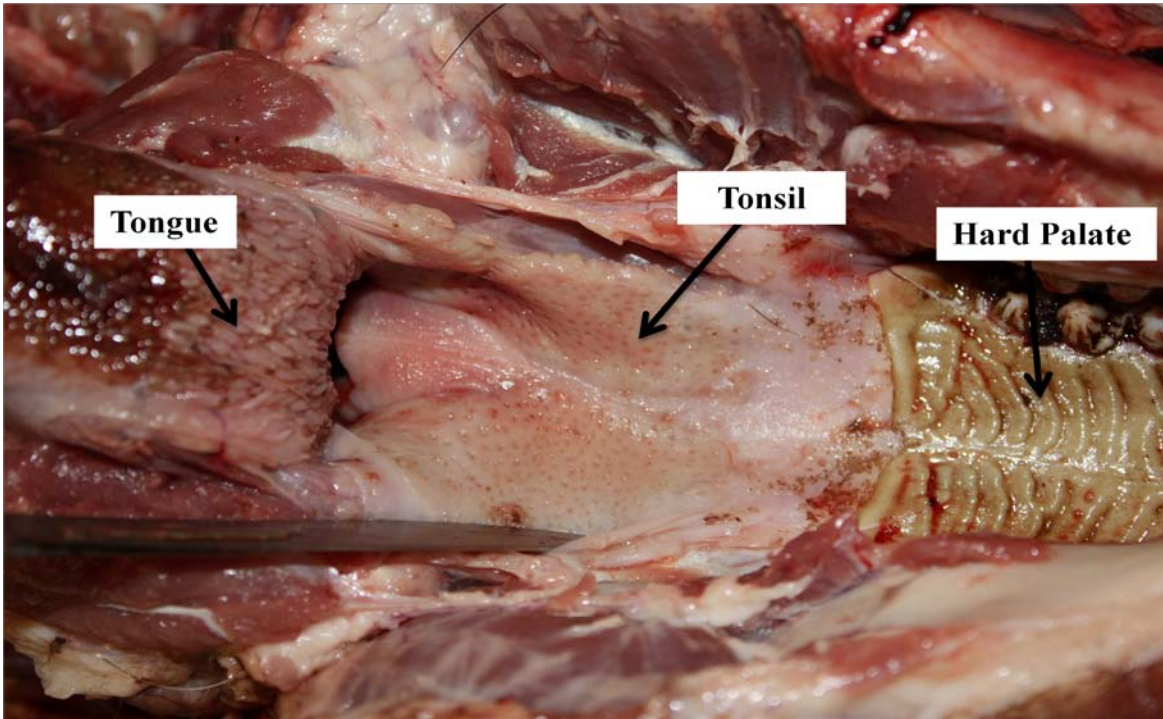




## Appendix H: Feral Swine Necropsy

### Removal of the tonsils:

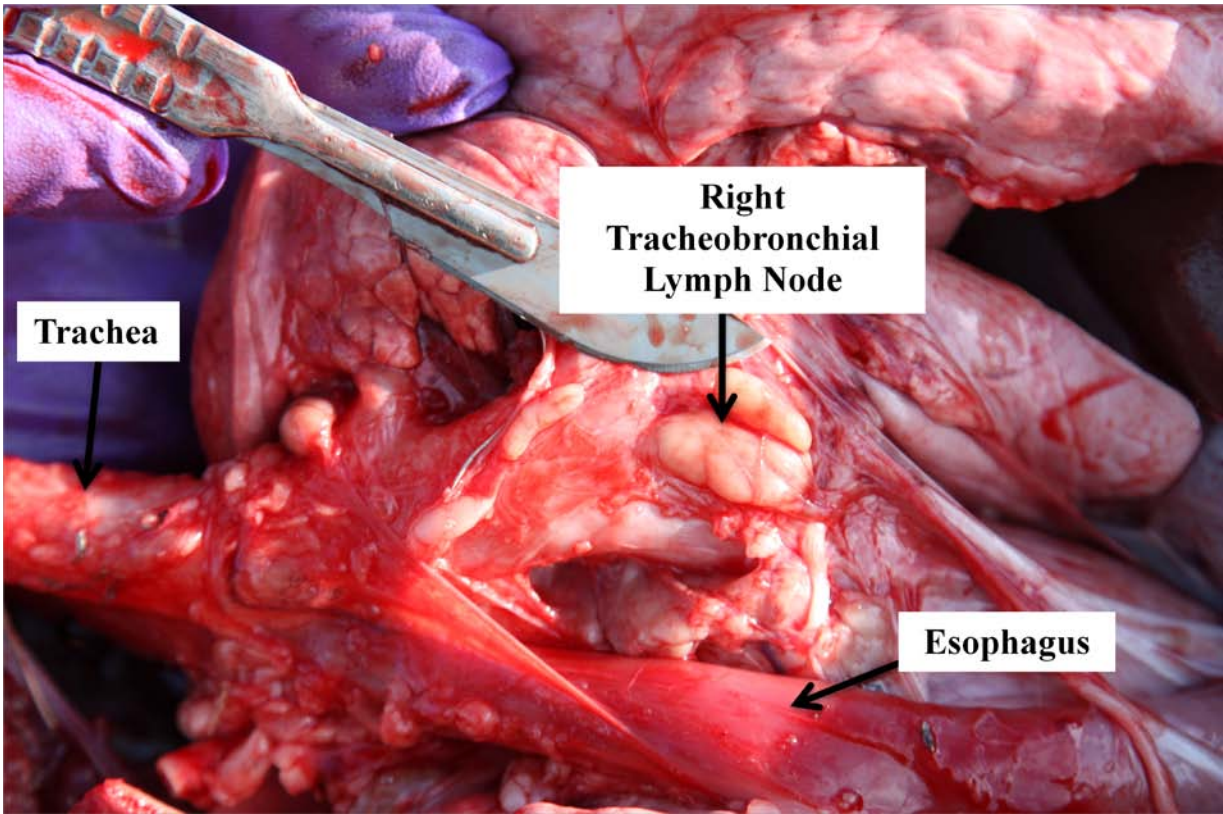
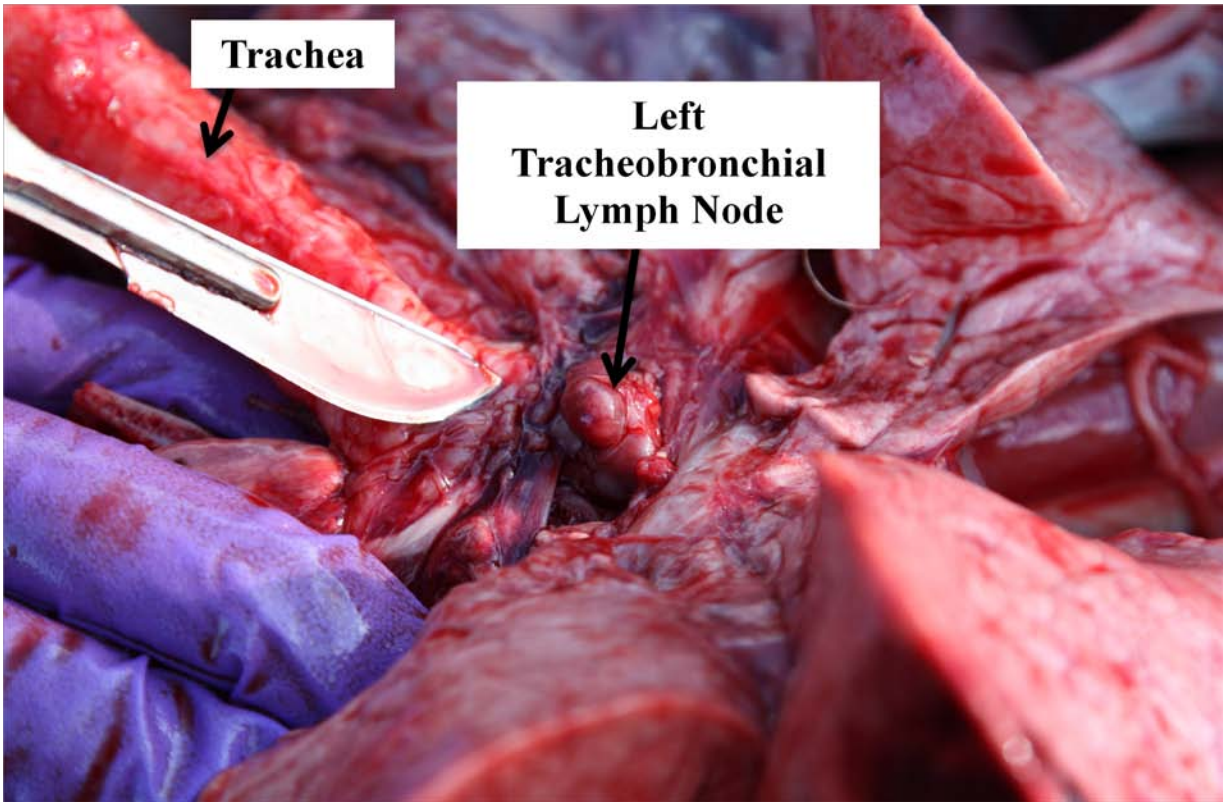
To remove the tonsils, cut along both sides of the mandible to free the tongue. Pull the tongue back towards the tail. The tonsils make up almost the entire soft palate directly behind the hard palate and are flat, large, and finely pitted. Carefully extract both tonsils.



### Removal of the thoracic lymph nodes and tissues:

Expose the chest cavity by cutting the ribs along the sternum. Visually examine and palpate the lungs for firmness, lumps, or lesions. Collect samples for culture and histopathology if any abnormal tissue is observed, and look for lesions on the wall of the chest or elsewhere in the chest cavity for abnormalities. If in doubt, submit the tissue to the laboratory to decide.

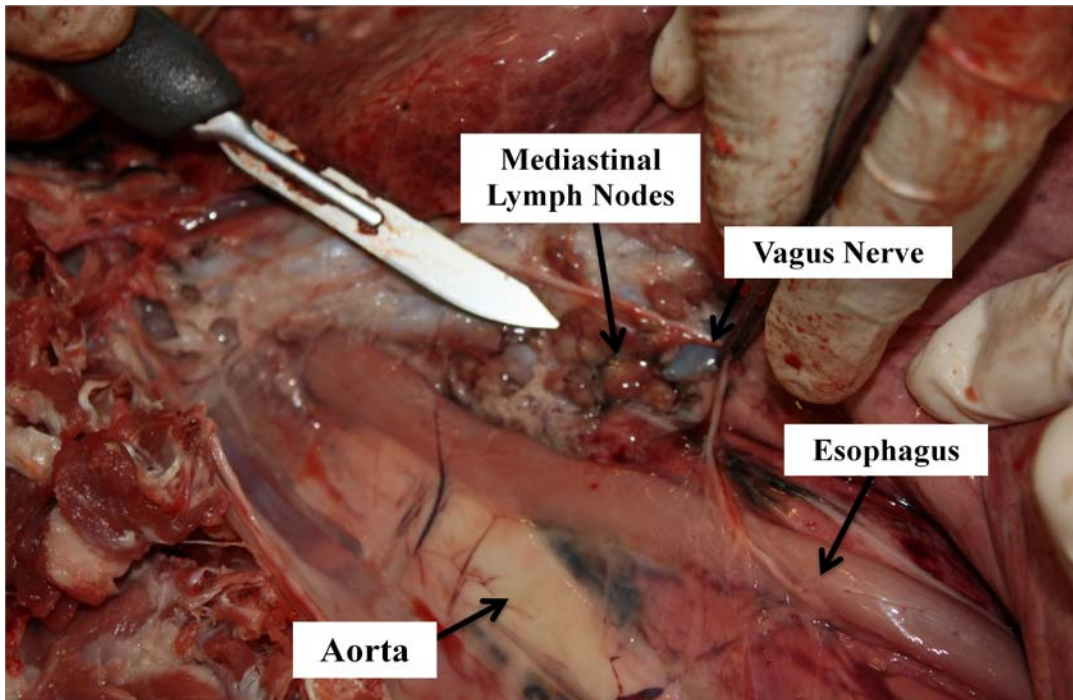
Identify where the trachea branches into the right and left bronchii. The tracheobronchial lymph nodes are located where the bronchii branch. The aortic arch surrounds the left tracheobronchial lymph node and is usually the more obvious of the two. An alternate method to finding the tracheobronchial lymph nodes is to cut down the trachea to where the bronchii branch.





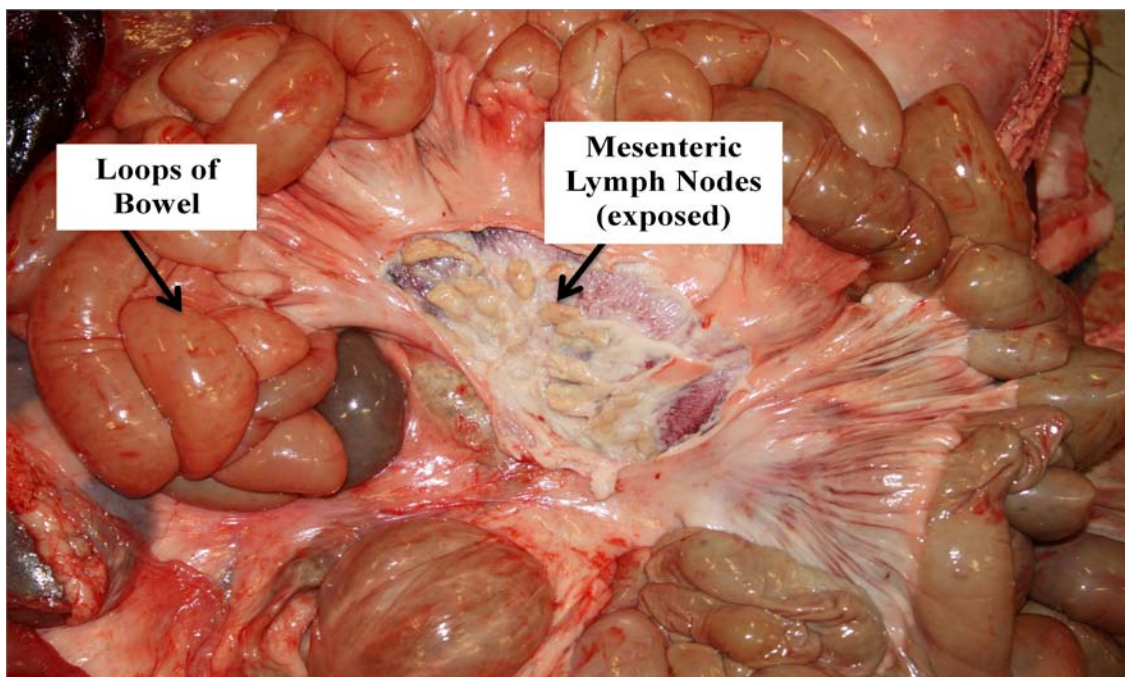
## Appendix H: Feral Swine Necropsy

The mediastinal lymph nodes are easiest to find with the animal lying on its left side. Lift up the lungs and the mediastinal lymph nodes can be identified as a series of small nodes between the esophagus and the vagus nerve underneath the lungs.



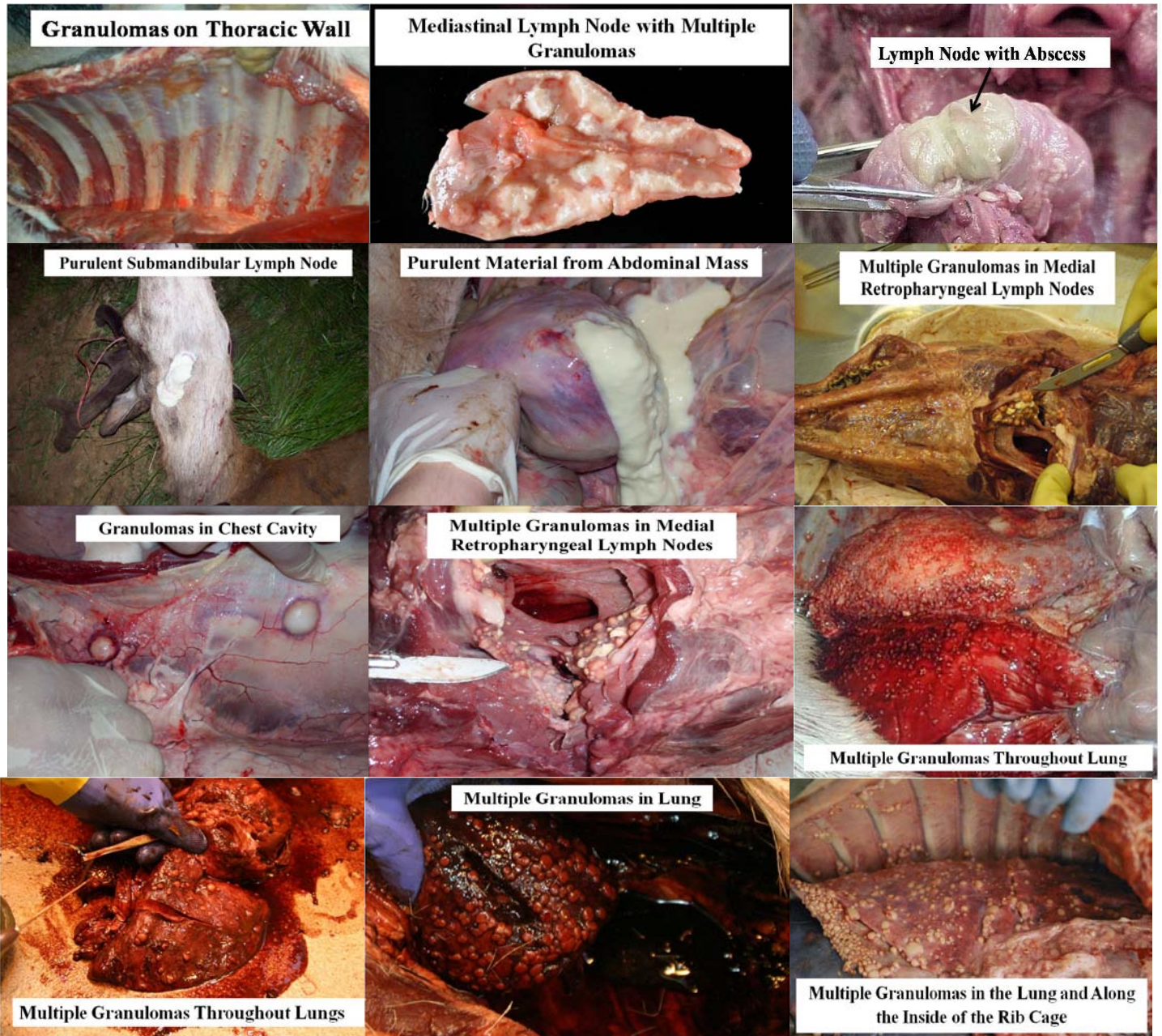
### Removal of the abdominal cavity lymph nodes:

Expose the abdominal contents by removing the flap of muscle that covers the abdominal cavity from the rib cage to the pelvis. The mesenteric lymph nodes are the only lymph nodes from the abdominal cavity to routinely submit to the laboratory. They are located within the thin, opaque mesentery that suspends the bowel. However, if the pig has a lot of fat they will be difficult to see.





## Cervid Lesions

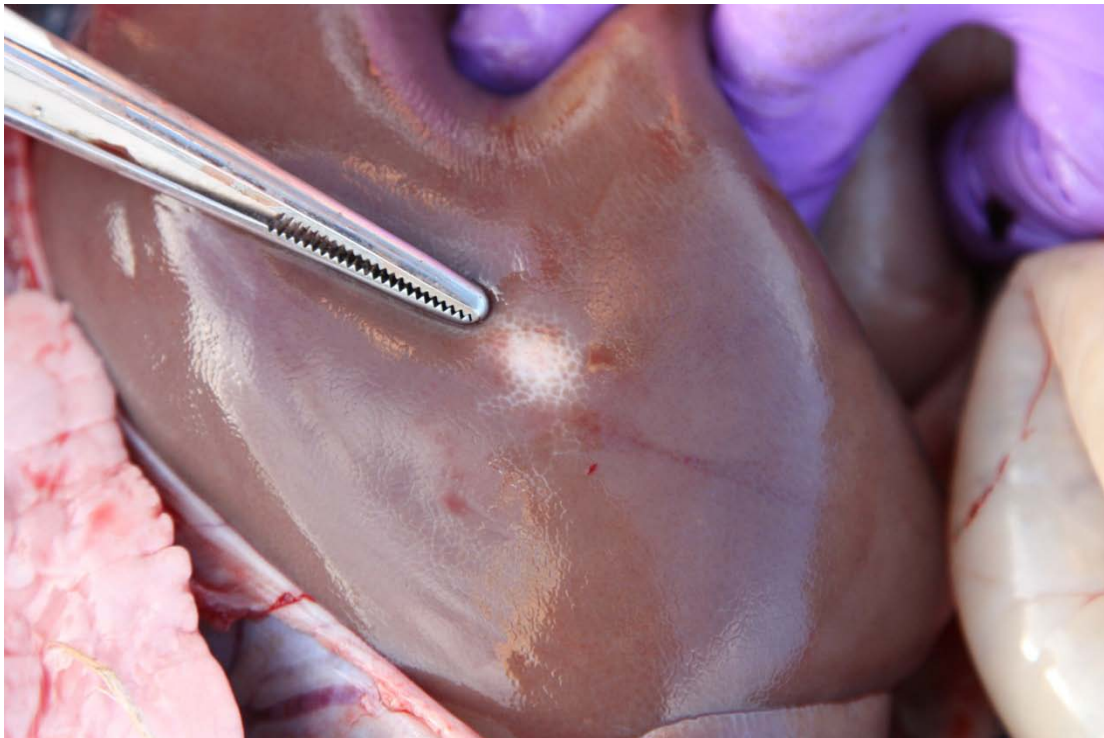




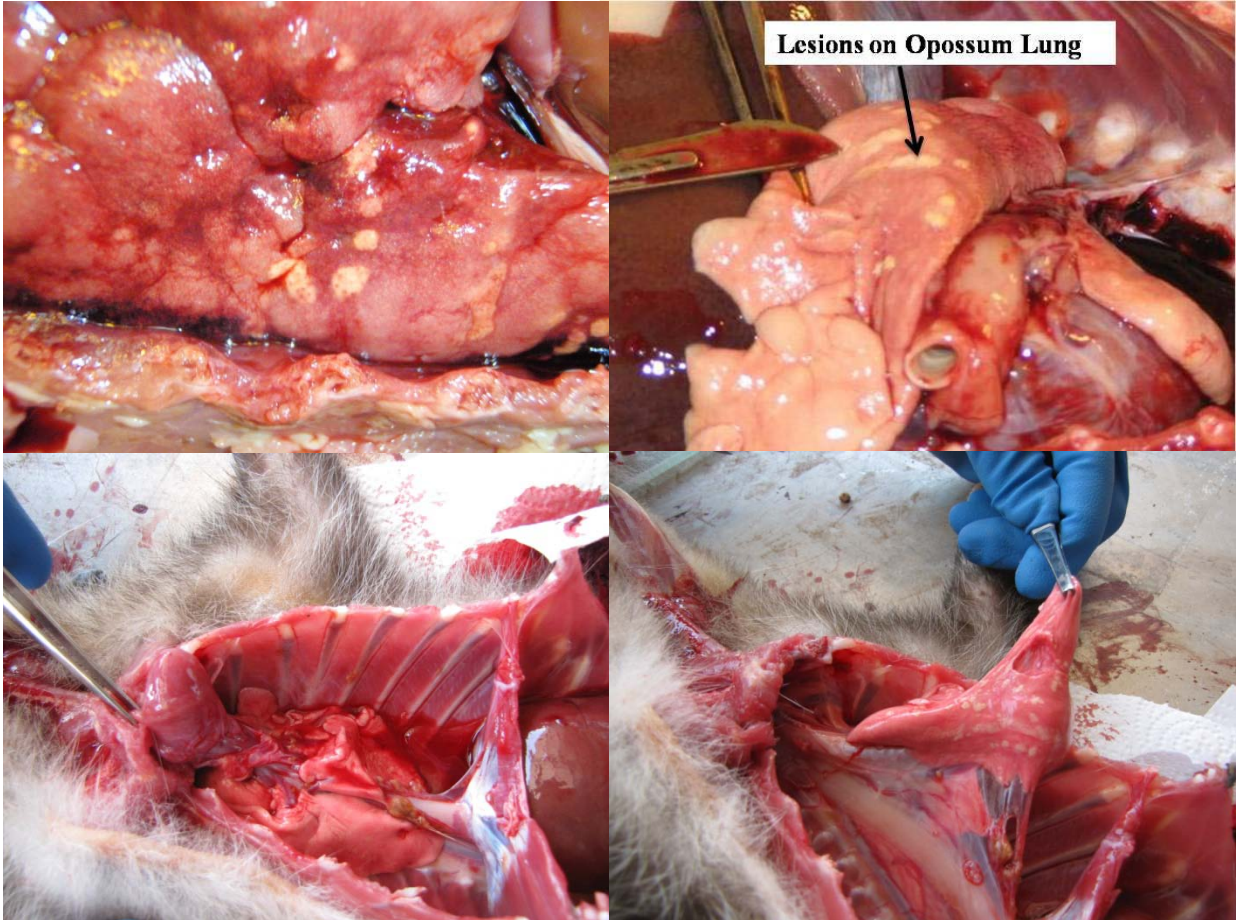
**Coyote Lesions**



**Feral Swine Lesions**






## Opossum Lesions





# Appendix J: Sample Wildlife Bovine Tuberculosis Surveillance Field Datasheet

USDA Wildlife Bovine Tuberculosis Surveillance		APHS		Page 1 of 1	
Collector: <u>Clark Kent</u>			Collection Site		
Agency: <u>USDA-WIS</u>					
Phone #: <u>260-1234</u> Email: <u>Clark.Kent@aphis.usda.gov</u>			<u>Somewhere</u>		
GPS location (In WGS 84 and decimal degrees): N <u>40.53675</u> W <u>105.36251</u>			County <u>Larimer</u>	State <u>CO</u>	Date collected <u>12/7/09</u>
<b>Sample Barcode</b>  1- <b>A00375195</b>	<b>Species (check one)</b> <input type="checkbox"/> Cervid (specify) _____ <input checked="" type="checkbox"/> Raccoon <input type="checkbox"/> Opossum <input type="checkbox"/> Feral swine <input type="checkbox"/> Canid (specify) _____ <input type="checkbox"/> Rodent (specify) _____ <input type="checkbox"/> Other (specify) _____	<b>Samples Collected (cross out lymph nodes or tissues not collected)</b> <b>Histo Culture</b> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> HEAD (parotid, submandibular, retropharyngeal, tonsil) <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> CHEST (tracheobronchial, mediastinal, lung) <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> ABDOMEN (mesenteric) <input type="checkbox"/> Check if serum sample is collected			
	<b>Age Class (check one)</b> <input type="checkbox"/> Juvenile <input checked="" type="checkbox"/> Adult <input type="checkbox"/> Unknown	<b>Sex (check one)</b> <input type="checkbox"/> Male <input checked="" type="checkbox"/> Female <input type="checkbox"/> Unknown	<b>Comments</b>  <b>Histo Culture OR <input checked="" type="checkbox"/> Check if no gross lesions observed</b> <input type="checkbox"/> <input type="checkbox"/> LESIONS (List tissues and describe):		
<b>Sample Barcode</b>  1- <b>A00375183</b>	<b>Species (check one)</b> <input type="checkbox"/> Cervid (specify) _____ <input type="checkbox"/> Raccoon <input checked="" type="checkbox"/> Opossum <input type="checkbox"/> Feral swine <input type="checkbox"/> Canid (specify) _____ <input type="checkbox"/> Rodent (specify) _____ <input type="checkbox"/> Other (specify) _____	<b>Samples Collected (cross out lymph nodes or tissues not collected)</b> <b>Histo Culture</b> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> HEAD (parotid, submandibular, retropharyngeal, tonsil) <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> CHEST (tracheobronchial, mediastinal, lung) <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> ABDOMEN (mesenteric) <input type="checkbox"/> Check if serum sample is collected			
	<b>Age Class (check one)</b> <input checked="" type="checkbox"/> Juvenile <input type="checkbox"/> Adult <input type="checkbox"/> Unknown	<b>Sex (check one)</b> <input checked="" type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Unknown	<b>Comments</b>  <b>Histo Culture OR <input checked="" type="checkbox"/> Check if no gross lesions observed</b> <input type="checkbox"/> <input type="checkbox"/> LESIONS (List tissues and describe):		
<b>Sample Barcode</b>  1- <b>A00045847</b>	<b>Species (check one)</b> <input checked="" type="checkbox"/> Cervid (specify) <u>white-tailed deer</u> <input type="checkbox"/> Raccoon <input type="checkbox"/> Opossum <input type="checkbox"/> Feral swine <input type="checkbox"/> Canid (specify) _____ <input type="checkbox"/> Rodent (specify) _____ <input type="checkbox"/> Other (specify) _____	<b>Samples Collected (cross out lymph nodes or tissues not collected)</b> <b>Histo Culture</b> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> HEAD (parotid, submandibular, retropharyngeal, tonsil) <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> CHEST (tracheobronchial, mediastinal, lung) <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> ABDOMEN (mesenteric) <input type="checkbox"/> Check if serum sample is collected			
	<b>Age Class (check one)</b> <input type="checkbox"/> Juvenile <input checked="" type="checkbox"/> Adult <input type="checkbox"/> Unknown	<b>Sex (check one)</b> <input checked="" type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Unknown	<b>Comments</b>  <b>Histo Culture OR <input checked="" type="checkbox"/> Check if no gross lesions observed</b> <input type="checkbox"/> <input type="checkbox"/> LESIONS (List tissues and describe):		
Date Samples Shipped to Testing Lab: <u>12 / 8 / 09</u> # of animals sampled: <u>3</u>			NVSL ACCESSION #		
<input checked="" type="checkbox"/> Check here if collector info is same as submitter Name of Submitter: _____ Phone # of submitter: _____					
Revision 9-23-09					

# Appendix K: Sample 10-4 Form

FORM APPROVED OMB NUMBER 0579-0090

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE NATIONAL VETERINARY SERVICES LABORATORIES P.O. BOX 844 1600 DAYTON AVENUE AMES, IOWA 50010 (515) 663-7212				<b>INSTRUCTIONS:</b> Use a separate form for each species and each owner/broker. See instructions for completing VS FORM 10-4 for definitions (Item 12) and instructions for identification (Item 20).		<b>PAGE</b> 1 OF 1																																																	
<b>SPECIMEN SUBMISSION</b>																																																							
<b>1. NAME OF SUBMITTER</b> Clark Kent, NVSL ID - COKEN				<b>2. NAME OF OWNER</b> NA																																																			
<b>MAILING ADDRESS</b> (Street, City, State, and Zip Code) 1234 Superman Hwy Kryptonite, CO 80320				<b>CITY</b> _____		<b>STATE</b> _____																																																	
<b>Phone No.</b> 970-266-1234 <b>FAX No.</b> 970-266-1235				<b>3. LOCATION OF ANIMALS</b> <b>COUNTY</b> multiple (see datasheets)		<b>STATE</b> CO																																																	
<b>4. PAYMENT METHOD</b> (Check applicable item and provide information) <input type="checkbox"/> USER FEE ACCOUNT NO.: NA <input type="checkbox"/> MC/VISA NO.: _____						<b>EXP. DATE:</b> _____																																																	
<input type="checkbox"/> CHECK/MONEY ORDER ENCLOSED (Made payable to "USDA" in U.S. Dollars)																																																							
<b>5. HERD/FLOCK SIZE</b> NA		<b>8. EXAMINATIONS REQUESTED</b> TB culture - 3 animals			<b>9. COLLECTED BY</b> USDA-WS																																																		
<b>6. NO. IN HERD/FLOCK AFFECTED</b> NA		Histopathology - 3 animals			<b>10. DATE COLLECTED</b> _____																																																		
<b>7. NO. IN HERD/FLOCK DEAD</b> NA					<b>11. AUTHORIZED BY</b> AVIC																																																		
<b>12. PURPOSE OF SUBMISSION</b> (Check one) (See instructions for definitions)						<b>13. COUNTRY OF ORIGIN/DESTINATION</b> USA																																																	
<input type="checkbox"/> General Diagnostic		<input type="checkbox"/> Surveillance		<input type="checkbox"/> Import		<input type="checkbox"/> Interstate Movement																																																	
<input type="checkbox"/> FAD/EP Diagnostic		<input type="checkbox"/> Developmental Research		<input type="checkbox"/> Export		<input type="checkbox"/> Other (specify) _____																																																	
<input type="checkbox"/> NVSL Intralab Diagnostic		<input type="checkbox"/> Reagent Evaluation		<input checked="" type="checkbox"/> TB		<b>14. REFERRAL NUMBER</b> _____																																																	
<b>15. PRESERVATION</b> (Check applicable item(s)) <input type="checkbox"/> None <input checked="" type="checkbox"/> Ice Pack <input type="checkbox"/> Dry Ice <input checked="" type="checkbox"/> Formalin <input type="checkbox"/> Borax <input type="checkbox"/> Alcohol <input type="checkbox"/> Other (specify) _____																																																							
<b>16. SPECIMENS SUBMITTED</b> (Check applicable item(s))						<b>17. TOTAL NUMBER OF SPECIMENS SUBMITTED</b> 14																																																	
<input type="checkbox"/> Blood		<input type="checkbox"/> Feces		<input type="checkbox"/> Parasite		<input type="checkbox"/> Serum																																																	
<input type="checkbox"/> Culture		<input type="checkbox"/> Feed		<input type="checkbox"/> Plant		<input type="checkbox"/> Soil																																																	
<input type="checkbox"/> Extract		<input type="checkbox"/> Milk		<input type="checkbox"/> Semen		<input type="checkbox"/> Swab																																																	
				<input checked="" type="checkbox"/> Tissue		<input type="checkbox"/> Whole Bird																																																	
				<input type="checkbox"/> Urine		<input type="checkbox"/> Fetus																																																	
				<input type="checkbox"/> Water																																																			
<b>18. SPECIES OR SOURCE</b> (Check one)						<b>19. NUMBER OF ANIMALS SAMPLED</b> 12																																																	
<input type="checkbox"/> Cattle		<input type="checkbox"/> Goat		<input type="checkbox"/> Environment		<input type="checkbox"/> Chicken																																																	
<input type="checkbox"/> Swine		<input type="checkbox"/> Horse		<input type="checkbox"/> Reagent		<input type="checkbox"/> Bison																																																	
<input type="checkbox"/> Sheep		<input type="checkbox"/> Donkey		<input type="checkbox"/> Pet Bird		<input type="checkbox"/> Cat																																																	
				<input type="checkbox"/> Dog		<input type="checkbox"/> Deer																																																	
				<input type="checkbox"/> Elk		<input checked="" type="checkbox"/> Other (specify) (ATTACHED)																																																	
				<input type="checkbox"/> Fish																																																			
<b>20. IDENTIFICATION</b> (See instructions)																																																							
<table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th>Sample ID</th> <th>Animal ID/Breed</th> <th>Age</th> <th>Sex</th> </tr> </thead> <tbody> <tr> <td colspan="4" style="text-align: center;">SEE ATTACHED</td> </tr> <tr><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td></tr> </tbody> </table>				Sample ID	Animal ID/Breed	Age	Sex	SEE ATTACHED																				<table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th>Sample ID</th> <th>Animal ID/Breed</th> <th>Age</th> <th>Sex</th> </tr> </thead> <tbody> <tr><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td></tr> </tbody> </table>				Sample ID	Animal ID/Breed	Age	Sex																				
Sample ID	Animal ID/Breed	Age	Sex																																																				
SEE ATTACHED																																																							
Sample ID	Animal ID/Breed	Age	Sex																																																				
<b>21. ADDITIONAL DATA</b> (History, clinical signs, post mortem findings, remarks, tentative diagnosis, etc. Use additional sheets if necessary.)																																																							
<b>22. SIGNATURE OF SUBMITTER AND DATE</b> _____																																																							
<b>NVSL USE ONLY</b>																																																							
<b>CONDITION</b> _____		<b>PRIORITY</b> _____		<b>DISTRIBUTION</b> _____		<b>RECEIVED BY</b> _____																																																	
						<b>NVSL ACCESSION NO</b> _____																																																	

VS FORM 10-4 (JULY 97)