Appendix H

BIGHORN SHEEP NECROPSY PROTOCOL

Introduction. Anytime a bighorn sheep dies naturally or is euthanized for management reasons, a thorough necropsy should be performed. Preferably, the entire carcass would be shipped to the Wyoming State Veterinary Laboratory (WSVL) so the necropsy can be conducted under controlled conditions (arrangements for delivery should be made with Hank Edwards of WGFD (307) 745-5865 or directly with WSVL (307) 742-6638). At times it is nearly impossible to deliver an intact carcass. When this occurs, trained personnel should perform field necropsies. This appendix is meant to serve as a check list and reminder of the tissues that need to be collected on field necropsies of bighorn sheep.

Methods. Please follow and fill out the necropsy form provided (additional forms available from Walt Cook at WSVL). Relevant animal information should be noted (location, disease history etc.). When possible, the animal can be weighed. Hair coat quality should be subjectively estimated. The hair coat should be thoroughly examined, and any external parasites noted and collected in a container filled with ethyl alcohol. Body muscle condition can be subjectively estimated on a scale of 0-5 (0=very poor; 5=excellent condition). Incisions can be made along the back at 1) tail base 2) cranial to hind quarter and 3) above the shoulder. Body fat is scored as follows: 0= no fat seen, 5= fat seen at 1 only, 10= fat seen at 1 & 2, 15= fat seen at all 3 locations. A subjective body condition can also be assigned (excellent, very good, good, fair, poor, very poor, emaciated).

The animal will then be opened per normal necropsy technique. The amount of fat may be measured (in mm) on the heart, kidneys, omentum and xiphoid. A femur may be cracked and the bone marrow examined for color and texture and taken for histology. Standard tissues should be sampled for histology; tissues need to be placed in a leak-proof container with plenty of 10% formalin (these are called “fixed” tissues). Be sure to sample at least 5 different areas of the lung. Any abnormal appearing tissues should be sampled for further evaluation at WSVL. A description of the abnormal tissue should be included on the necropsy form. Photographs of lesions/abnormalities are also useful.

All bighorn sheep should have fresh lung taken for virus isolation and microbiological culture. Fresh tissues should be sampled using sterile or very clean technique. This is best accomplished by dipping clean instruments in 70-90% ethyl alcohol and burning the alcohol off over a flame. Do not touch any of the tissues directly with your hands, but place the tissue in a Whirl-pak® bag with the forceps. At least two separate bags containing lungs should be provided. Do not add any preservatives or other material to the bag; only one tissue sample should be placed in each bag. Collapse the bag to expel all air and hold the tabs with your fingers and tightly turn the top of the bag at least four times. Bags, tubes, and containers need to be labeled with indelible ink (e.g. Sharpie® pens). All bighorn sheep should also have their tonsils sampled. This is done by swabbing the tonsil with a sterile culture swab and placing the swab in a Port-A-Cul™ media tube (available from Hank Edwards).

Blood may be taken for complete blood counts (CBC), serum chemistries, trace mineral analysis, and/or serologic testing for antibodies to relevant diseases. Blood can be taken directly from the heart or from the jugular vein. Ideally, each sheep will have 2
red top blood tubes filled to the top (1 for serology, 1 for trace minerals) and 1 green top (for selenium). If zinc is a concern, a royal blue top tube will need to be filled in place of one of the red top tubes (these tubes are very expensive and not routinely used-contact Hank Edwards or WSVL if you need these tubes). If CBCs and blood chemistries will be performed, a purple top tube will need to be filled as well. If the animal has been dead for more than a couple hours, it will be extremely difficult to obtain quality blood samples.

The lower middle two incisors (I1) may be pulled for cementum annuli aging; alternatively ages can be estimated by tooth replacement and wear and/or horn ring counts. Fecal samples need to be taken to determine parasite loads; this can be accomplished by placing 5-6 inches of the terminal rectum containing at least 20 pellets in a Whirl-pak® bag. Using long Q-tip type swabs, swab deep around the inside of both ears and put the swabs in Whirl-pak® bags. A piece of the ear should also be put into formalin for microscopic examination for mites. Using a culturette, swab deep in the nose and return swab to its housing. Be sure to crush the bottom to release the preservative. All tissues should be kept cool, but not allowed to freeze.

The entire gastrointestinal tract should be opened and thoroughly examined, and the type and number of macroscopic parasites recorded. Internal parasites may be preserved in 5% formalin (mix 10% formalin with an equal part water). Other tissues (liver, kidney, brain, rumen contents) should be saved and stored for future testing (e.g. toxicology and mineral analysis). The uterus of ewes should be opened and the number, sex, weight, crown-rump and crown-nose length of all fetuses recorded. If the herd has had reproductive problems, fetal tissues should be taken fresh and for histology.

We recognize that this protocol is somewhat idealistic. In field situations available personnel may not always have the instruments, expertise, or time to conduct thorough necropsies. When this is the case, remember that it is most important to sample lungs (fresh and fixed) and to provide fresh liver if minerals (especially selenium) are a concern.

Shipping. Tissues need to be shipped to WSVL as soon after the necropsy as possible (this is very important for fresh tissues and blood samples). It is preferable for samples to be personally delivered to WSVL. If you choose to ship fresh samples or blood via the US Mail, UPS, or Fed Ex special regulations apply. All samples must be double bagged, the shipping carton must be insulated and protected by an outer fiberboard box. Extra spaces in the box should be filled with newspaper or paper towels. Add frozen ice packs to the package and place all paperwork in a separate plastic bag and put it on top of the insulated container lid. It is best to avoid shipping over a weekend; keep the package in a refrigerator until Monday. If you have further questions, contact Hank Edwards or the WSVL at the numbers above or look on the web:
Bighorn Sheep Necropsy Form

Herd name/location __________________________ Animal id (if any) ______
Location: Township ______ Range ______ Section ______ or UTM ________________
WSVL Accession # ________________ Date of necropsy ____________

History/signs of disease prior to death:

Estimated Age ___________ Sex ___________ Weight (if available) ____________
Hair coat quality: Excellent Good Fair Poor Very poor
Species and number of external parasites: Collectected? ____________

Body muscle (0-5) _______ Back fat score: 0 5 10 15
Mm Fat on: Heart _______ Kidneys _____ Omentum _____ Xyphoid _______
Bone Marrow Color _______ Texture _______ Subjective Body Condition: ____________

Internal Exam Notes:

Species and number of internal parasites: Collectected? ____________

# Fetuses: _______ Weight: #1: _______ #2: _______ Sex: #1: _______ #2: _______
Crown-Rump: #1: _______ #2: _______ Crown- nose #1: _______ #2: _______

Tissues fixed:          Tissues taken for laboratory evaluation:  Others (list):  
Heart ______________  Fecal (parasitology) _______  
Liver ______________  Blood (red tops) x2 _______  
Spleen ______________  Blood (green top) _______  
Lung ______________  Blood (purple top) _______  
Tongue ______________  Teeth (both 11s) for aging ___  
Muscle ______________  Lungs ______________  
Kidneys ____________  Tonsil swab in Port-A-Cul ___  
Rumen ______________  Liver ______________  
Reticulum ___________  Kidneys ______________  
Omasum _____________  Brain ______________  
Abomasum ___________  Rumen contents _______  
Ileum ______________  Feces ______________  
Gonads ______________  Fat ______________  
Brain ______________  Nasal Swabs _______  
Pancreas ____________  Ear Swabs _______  
Ileocecal LN ________  Fetal Tissues Fresh:  
Bladder _____________  Kidney _______  
Bone Marrow ___________ Eyeball _______  

Fetal Tissues Fixed:  
Liver _______  Abomasum _______