## Rangifer Health & Body Condition Monitoring

# MONITORING PROTOCOLS

## LEVEL 2





CircumArctic Rangifer Monitoring and Assessment (CARMA) Network

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## INTRODUCTION

This "Protocols Level 2" document should be used together with the **Rangifer Health & Body Condition Monitoring Manual**. These monitoring protocols include several indicators of morphometrics, fatness, protein reserves, and prevalence of disease and parasites. Protocols for monitoring contaminants are included. **Monitoring at this level will require the presence of a veterinarian or trained/skilled biologist or technician to collect and process samples. It will also require partnerships with laboratories to analyze and assess samples for diseases, parasites and contaminants. Assessment of protein reserves would require partnership with biochemical researchers that have access to lab facilities.** 

We expect this level of monitoring will be implemented through regional wildlife agencies, and in partnership with the communities and co-management boards in the areas where collections take place.

If you have any suggestions for changes or improvements to this document please contact:

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## DATA RECORDING STANDARDS

Labels – All units MUST be <u>clearly labelled</u>. The following are a few examples:

- gram (g), kilogram (kg)
- millimetre (mm), centimetre (cm), millilitre (ml), litre (l)
- calendar dates (day/month/year)

Handwriting – Handwriting (including numbers) must be legible.

**Sample Identification** – Identification numbers for each animal collected should have a combination of alphabet letters and numbers. ID numbers MUST accompany ALL samples.

**Numbers** – To clearly distinguish the number one (1) from the number seven (7), always "cross" the vertical stroke of the number seven as shown: 7. If there is any doubt to whether the number is a 6 or a 9, underline it as shown:  $6 \ 9$ .

## **SHIPPING NOTES**

Formalin can be discarded after 4 days and samples can be shipped 'dry' with a formalin-wet cloth in the bottle.

Always OVER pack your samples for shipping. This means triple and quadruple bag them in plastic. Also, add paper towel or newspapers in-between bag layers. **There is always leakage!** 

## THE PROTOCOLS

If possible, animals should be shot in the neck to minimize damage to the tissues and organs that will be sampled using these protocols.

#### VITALS

Prior to collecting samples from the caribou or reindeer, the following important information should be recorded:

- collection number (If your jurisdiction has a collection tracking system, use numbers that are compatible with that system. For reference herds the collection number should include "carma-ipy- ....")
- herd
- date
- sex of animal
- hunter
- location of the harvest

**LABEL EACH ANIMAL** by attaching a tag through the ear and by writing collection # on the antler. **PHOTOGRAPH EACH ANIMAL** and place an object of known size (for example, a measuring stick, notebook or label tag) in the photo for scale. If possible, take one photograph of the head and one of the entire body.

## 1. AGE

## 1.1 Maturity

Categories of maturity for all levels of monitoring are based on tooth eruption and should be recorded as:

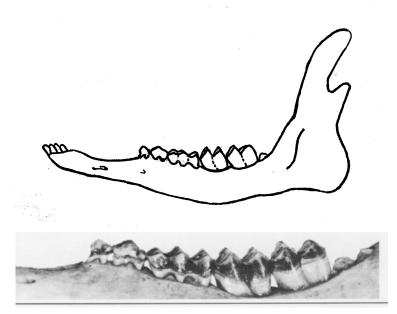
- calf (less than one year old)
- subadult (juveniles and yearlings: 1- 3 years old) (Figure 1)
- adult (>= 3 years old, may or may not be breeding at time of collection because reproductive pauses may occur in adult females) (Figure 2)

It is important to record maturity independent of knowing the cementum age, as it tests the validity of using 3 maturity categories rather than only two (calf vs. adult).

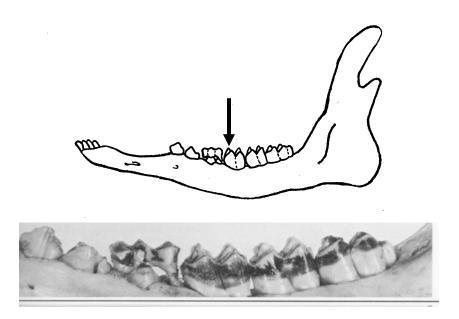
#### Age classification "calf – subadult – adult" by cheek tooth:

1.1.1 Calf teeth (< 1 year) are all milk teeth. They are obvious to even the most inexperienced observer.

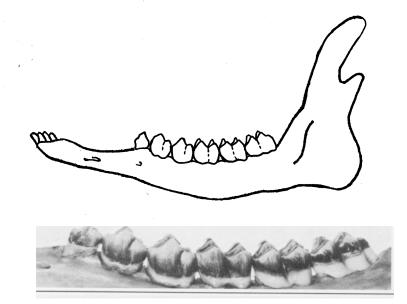
- 1.1.2 Subadults (between 1 and 3 years) have clearly erupting cheek teeth and often the first 3 cheek teeth are well worn because they are "milk" teeth (Figure 1). Sometimes premolars are seen in the process of being pushed out from below (Figure 2). If erupting teeth are present, the animal is NOT a mature adult, therefore can be classed as a "subadult".
- 1.1.3 Adult (> 3 years) have 6 adult cheek teeth. Absolutely nothing is erupting from the gum line. The only change over time will be tooth wear (Figure 3) and with increasing age, erosion of the entire tooth row down to the gum line.



**Figure 1.** Tooth eruption of sub adult (> 1 year old). Photo & diagram (without gum tissue) shows only 5 teeth (3 milk premolars and 2 adult molars), and the 6th is barely visible and would still be buried in gum tissue. Note first 3 cheek teeth are milk teeth and show some wear, third tooth from the left (P4) has 3 "peaks".



**Figure 2**. > 2 year old tooth eruption. This photo shows 6 teeth. The P2 & P3 teeth are adult, but P3 would be barely visible above the gum line, i.e., it is erupting, and the  $6^{th}$  (M3) has just come above the gum tissue. The best indicator of age here is the  $3^{rd}$  tooth, which is the well worn P4 milk tooth with 3 "peaks". Most importantly **P4 is being pushed out-of-the-way** by the erupting adult P4 coming up from underneath. Be careful; do not to mistake a large bodied juvenile for an "old" adult because of the extreme tooth wear on the almost finished P4 milk tooth.



**Figure 3.** > 3 year mature adult with full set of 6 adult cheek teeth, which are all well above the gum line (as indicated by the separation of dark and light zones on this example). With some experience, observers will have no problem distinguishing adult teeth from juvenile teeth.

## 1.2 Cementum age

Equipment needed:

- sharp knife or saw/axe
- dental elevator
- extractor or pliers
- labels
- small paper envelopes

The first incisors are used for cementum aging after being extracted from the lower jaw (mandible).

The lower jaw should be photographed and/or sketched and described to show the extent of tooth wear on the incisor and cheek teeth. The photographs and written descriptions will be used for validating age class assignments. In younger animals, they will be used to determine the timing of tooth eruption. After photographs have been taken, the lower jaw should be collected and labeled. Be careful when removing the jaw of young animals, the bones may be soft and easily cut.

#### Take morphological measurements (see below) BEFORE extracting teeth.

Extract the teeth using a dental elevator (Figure 4) to carefully but completely loosen the tissue around the entire tooth circumference. The tooth can then be pulled straight out with an extractor or similar tool such as pliers.



Figure 4. Dental elevators

Once extracted, store teeth in paper envelopes in a cool and dry place. Do not store in plastic as they may rot. If teeth are to be stored for several months or years, store them cleaned and frozen. If you do not have a lab facility in your jurisdiction where tooth can be sectioned and aged, it can be shipped to a commercial lab for aging (see Appendix 1 for contact information). Reliability of a commercial lab should be established, preferably with known age samples. Equipment list for mandible and tooth processing:

- sharp knife
- labels
- plastic locking cable
- pencil
- small paper envelopes for storing teeth
- freezer for long term storage
- camera for photographing tooth wear in lab;
- small measurement calipers
- sharp knife
- dental elevator
- freezer

Also need access to tooth sectioning lab.

## 2. MORPHOLOGICAL MEASURES

## 2.1 Mandible and incisor tooth

- a. Use 2.5 X 4" poster board shipping tags, or equivalent size tags.
- b. Label these shipping tags using pre-printed self-adhesive address labels, which when printed are then stuck onto the 2.5 X 4" poster board shipping tags (Figure 5)
- c. The self-adhesive address labels can be pre-printed with :
  - ID numbers (generated in MS Word by creating a new Caption with auto-numbering)
  - A "ruler" along the edge of the label by creating a table of ½ cm columns.
  - Include space for vitals: hunter name, community, herd, date, location, sex, reproductive / lactational status, hunter assessment of condition, depth of backfat using "ruler" - see Figure 2.
- d. Attach tags to jaws using plastic cable ties or sturdy wire.

$\left( \right)$	SEX: Male Female	
	PREGNANT?: Yes or No NURSING?: Yes or No	
	<b>CONDITION:</b> skinny not bad fat very fat	$\bigcirc$
	DEPTH of BACKFAT:cm	
	2    4    6    8	

(YOUR HERD) Jaw Collection	
ID: 001	
Date:	$\square$
Location:	
Return to: (agency/office location, phone number)	

Figure 5. Jaw tag with adhesive label

Lab Measurements:

- be sure all tissue is removed from the posterior edge of jaw before measuring (bone is fairly thin here – be careful not to cut into the bone).
- measure the jaw before extracting the teeth and note whether the jaw was dried or not.
- with small calipers, make measurements recorded to the nearest mm along the tooth margins at the gum line (ie. diastema measured from anterior margin of premolar at gum line to posterior margin of canine at gum line).

As illustrated in Figure 6, measure to nearest mm:

- diastema
- anterior jawbone (assess growth from birth to subadult)
- posterior jawbone (assess growth from subadult to mature adult)
- total jawbone
- height of enamel on anterior crest of m<sub>2</sub>
  - once measurements have been completed, extract the first incisor as described in the preceding section "cementum age".

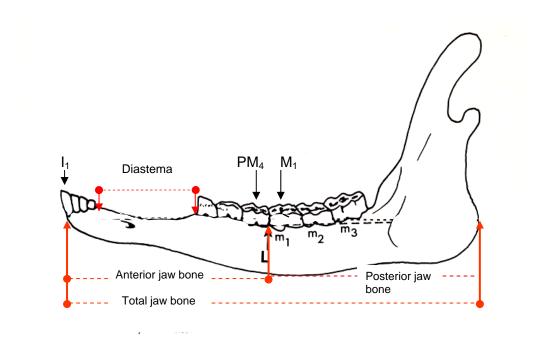


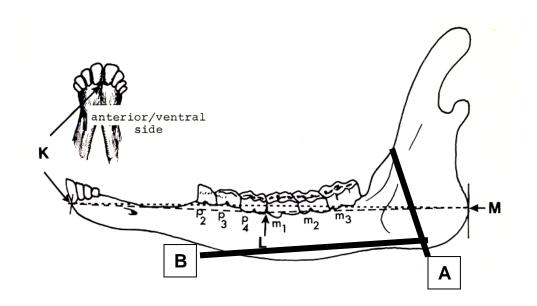
Figure 6. Diagram of jawbone boundaries (to nearest millimeter).

#### Jaw bone marrow (and connective tissue)

Marrow from the lower jaw can be extracted for analysis of water content, (thereby providing a measure of fat content). However, the metatarsus marrow gives a more direct relationship with body fat, and is therefore the best option for fat assessment based on marrow. Metatarsus marrow is also easier to remove than jaw marrow (See "metatarsus marrow measurement" for lab method).

#### Removal of jaw bone marrow (and connective tissue)

Figure 7 demonstrates the approach that CARMA is suggesting for removing jaw bone marrow. The intent is not to gather all of the bone marrow, but rather a large enough portion to allow for a calculation of the proportion of water in the sample (wet weight versus dry weight). Be careful to exclude any bone fragments from the inner jaw – you will be able to feel fragments with your fingers (Figure 8).



**Figure 7**. Directions for sawing jaw for marrow extraction. Saw completely through the jaw at line A. Saw along the outside of the jaw at line B and crack open with a sturdy knife or screwdriver.



Figure 8. Bone fragments from jaw bone marrow

## 2.2 Body size – adult

Equipment list:

- measuring tape
- large calipers

All measurements should be recorded to the nearest centimeter. Use new Cloth measuring tapes, as they tend to stretch over time. Plastic tapes do not stretch but may stiffen or crack in cold weather field conditions. Do measurements by keeping the measuring tape as close as you can to the body ("fluffy" hair may influence measurements, for the girth or circumference measurements particularly). Measure the metatarsus while the leg is still on the animal and before skinning the animal (Figure 9).

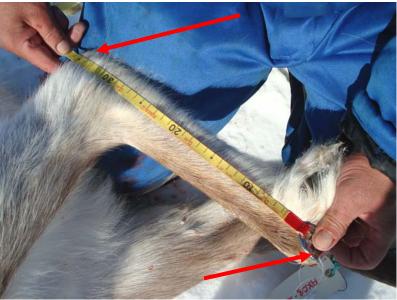
Photograph the animal. Include a label and an object of known size for scale.

Photograph:

- a. entire body
- b. head only, with clear view of antlers

#### Measure Metatarsal length:

Use tape measure or large calipers - length from the proximal end of the calcaneous to the coronary band (bend the hoof back as in Figure 9).



**Figure 9.** Metatarsus measurement – red arrows indicate the calcaneous and the coronary band

#### Measure antler size:

Measure main beam length, from base to main beam tip on the lateral side (as in Figure 10) AND total number of points on each side of the rack.

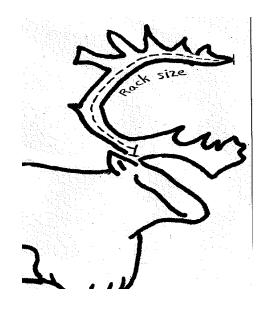


Figure 10. Antler size

Make the following measurements (as described in Figures 11-14).

- total body length
- tail length
- chest girth
- neck
- hind foot
- shoulder height

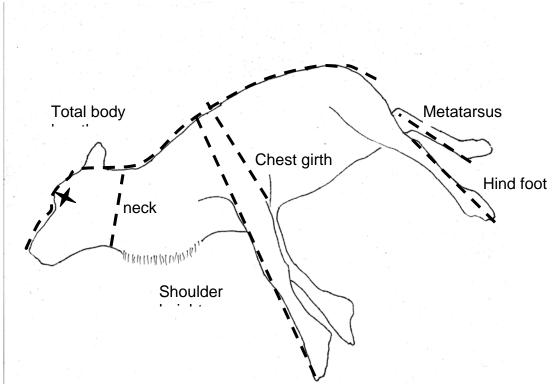


Figure 11. Body size – adult

**Total length**: Length along the spine from the tip of the snout (Figure 12) to the tip of the last bone in the tail. Keep the tail bent down and the head-neck at a 90° angle. If the tail or head is extended/stretched-out, this will alter the final total length measurement.



**Figure 12**. Measure of total body length. Arrow indicates starting point on snout.

**Tail length:** Lift tail straight out from the rump (Figure 13) and measure from base to the last bone in the tail.



Figure 13. Tail length

**Neck circumference**: Circumference of the neck immediately behind the mandible – measure the narrowest part of the neck

Heart (or Chest) girth: Circumference of the body immediately behind the shoulder and elbow

**Shoulder height**: Due to the variation in hoof length between seasons, CARMA recommends 2 different measurements: Shoulder height 1. Distance along the outer body from the spine at the shoulder hump to the distal end of the metatarsus – bend the hoof back as in Figure 14.



Figure 14. Shoulder height 1. Spine to end of metatarsus

Shoulder height 2. Distance along the outer body from the spine at the shoulder hump to tip of hoof with leg/hoof extended straight as in Figure 11.

**Hindfoot length**: Use large calipers to measure the (straight line) length from the back of the ankle to the tip of the hoof (Figure 11)

## 2.3 Body size – fetus

Handling of a fetus may require special permission from community members. Standard fetal measurements include weight, crown-rump (Figure 15) and transorbital (shortest distance between top of each eye socket), along with vitals.



Figure 15. Crown-rump measurement

## Additional Fetus Measurements:

Growth Rates: If fetal growth rates are of interest for time-series study, you can record vitals and take measurements from fetus collections in the same manner as adult measurements (weight, total body length, metatarsus, hind foot length, shoulder length, neck circumference and chest girth).

NOTE: testing for protein balance of cow-fetal pairs will require sampling of blood serum + muscle from the fetus. See section on protein balance.

## 2.4 Body Mass

Equipment list: (all scales should be calibrated prior to every use)

- tripod (large enough that entire caribou is off the ground when being weighed.)
- large mass scales
- OR platform scales (no tri-pod needed if using platform scales)
- pulley if using a tri-pod
- sling, rope
- several suspension scales (including smaller scales for head, hide and warble weights)

## Direct measurement

**Total body mass**: weigh the entire animal before skinning and sampling (Figure 16). You may need to add 1 kg for lost blood / rumen contents. A portable tripod and scale with pulleys, or large platform scale are useful.



**Figure 16**. A portable tripod and scale with pulleys are helpful for weighing. The animal in this photograph is being lowered to the ground after weighing.

**Weigh Head**: Cut between atlas (1st vertebrae) and cranium so neck stays with body. Remove head and weigh with antlers (if antlers are present).

#### Weigh Antlers

Remove and weigh the portion that is annually shed (i.e. don't include pedicles) Pull any velvet off before weighing.

**Weigh Cold Carcass**: This is the weight of the animal without its head, no skin, no viscera (including kidneys, lungs, heart etc) and no lower legs (Metacarpus / Metatarsus). (Figure 17)

Note: This MUST be taken BEFORE muscle tissue or bone removal.



Figure 17. Cold carcass weight

Weigh Skin/Hide: Weigh with most fat removed. For spring collections, weigh the hide along with warble larvae, and using a small scale weigh a representative sample of 10 warble larvae. Estimate the total number of warble larvae in the hide (or do a complete count).

**Weigh Rumen**: Body weight will vary with rumen fill, so extract and weigh full rumen (Figure 18) Put the rumen into a tub, cut it open, empty the contents into the tub, and mix thoroughly. Fill 1 litre Nalgene bottle with homogenous rumen and freeze for later calibration work. Discard the rest after weighing empty rumen and reticulum.

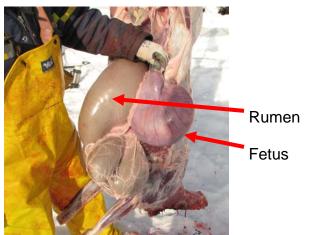
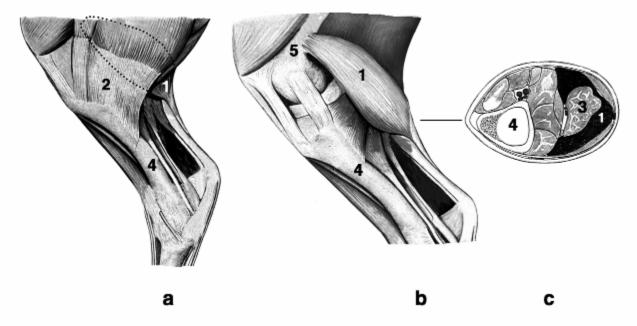


Figure 18. Rumen (left side of photo) and fetus inside uterus

**Weigh Fetus**: Remove entire uterus when eviscerating a cow, and weigh entire uterus along with fetus and amniotic fluid, then cut umbilical cord, remove fetus and weigh fetus alone.

If other methods are used for measuring body weight, then details should be noted (e.g. "animal was not skinned" for cold carcass weight). Whenever total body mass is recorded, the cold carcass weight should also be recorded in order to determine the relationship between carcass and total body weight.

Optional: An <u>index of body protein</u> reserve could be developed using indicator muscles such as the gastrocnemius (Figure 19) or the peroneus (Figures 20 -22). The following diagrams from Popesko (1979) show the location of the gastrocnemius muscle on the rear leg. If the gastrocnemius is used, collect the full extent of the tendon along with the muscle.



**Figure 19**. Identification of the gastrocnemius in the bovine hind limb; modified from Popesko (1979).

1 - gastrocnemius; 2 - gluteobiceps; 3 - flexor digitorum superficialis; 4 - tibia; 5 - femur.

(a) Medial view of the hind limb, with superficial fat and fascia removed. The gastrocnemius, indicated by dotted line, is largely covered by the gluteobiceps.

(b) Medial view of the hind limb, with the distal portion of the gluteobiceps removed.

(c) Transverse section of the hind-limb near the proximal end of the tibia; the gastrocnemius, indicated by dark shading, "surrounds" the flexor digitorum superficialis, a smaller muscle continuous with the large common calcaneal tendon.

#### **REFERENCES:**

Popesko, P. (1979). *Atlas of Topographical Anatomy of the Domestic Animals.* Vol. 1, Third Edition. London: W. B. Saunders Co.

Adamczewski, J. Z. 1995. Digestion and body composition in muskoxen. Ph.D. thesis, University of Saskatchewan, Saskatoon, SK. 138 pp. Use caution when extracting the gastrocnemius and/or the peroneus muscles, so that the entire muscle is taken. This is particularly important for the peroneus, as it is small and weight will not be representative if some muscle tissue is left behind.

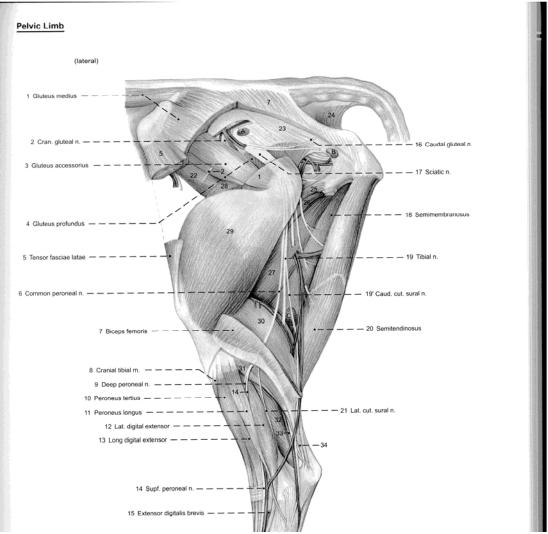


Figure 20a. Peroneus in place on lower leg (tibia)

**Figure 20b.** Peroneus cut away at distal end, in tact at proximal end



**Figure 21**. Peroneus removed from lower leg (tibia). Distal end is at bottom left corner of the photograph

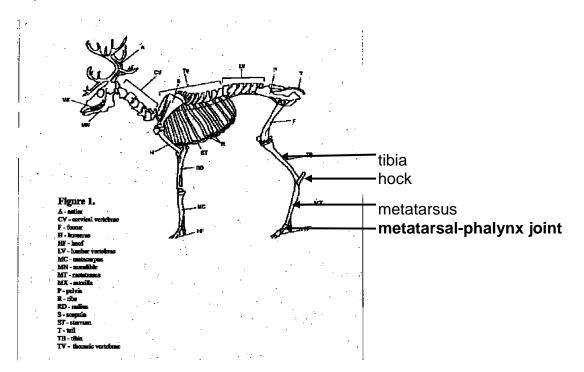


**Figure 22**. Peroneus tertius in place (No. 10 in this figure from: Budras et al. 2003. *Bovine Anatomy*, 1st edition)

## 2.5 Metatarsus size

#### Removal of metatarsus from carcass

Skin the lower back leg and cut the foot off at the metatarsal-phalynx joint (Figure 23).



#### Figure 23. Rangifer skeleton

At the proximal end (closest to body of the caribou) of the metatarsal, there is a joint that is not readily seen. As you run your hand away from the hoof up the metatarsus towards the ankle/"hock", it will start widening close to the ankle/"hock" (Figure 24). About ½ or ¾ of a centimeter along the widened

section, with a sharp knife make a cut completely around the bone and as deep as you can through the tendons. If the cut is in the right spot, you can bend the joint over your knee and the joint will break cleanly. If you are a bit off the right spot, there are 3 small bones that may come off with the metatarsus. If they aren't attached too tightly, try to take them off in the field using a sharp knife. This is easier to do when the bone is fresh, but they can be removed in the lab.

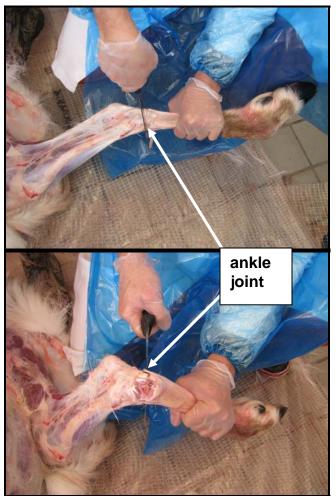


Figure 24. Removal of metatarsus

The hoof is easily removed from the metatarsus by cutting through the ligaments at the top of the hoof (Figure 25).



Figure 25. Removal of hoof

Ensure the metatarsus has no extra small knuckle bones at the proximal end (Figure 26) and clean off any excess tissue. Some bits of tendon will probably still adhere to the ends.

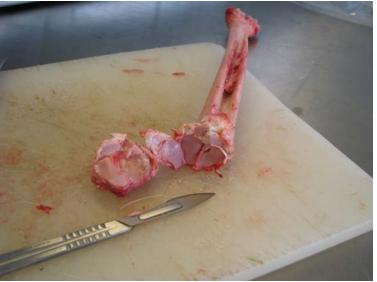


Figure 26. Metatarsus with small knuckle bones cut away

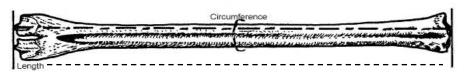
#### Metatarsus Measurements

Weigh the fresh metatarsus (to the nearest mg). Measure the length of the metatarsus using calipers (Figure 27).



Figure 27. Length of the metatarsus

In the middle (measure from each end to find middle – Figure 28) of the metatarsus; measure (to the nearest mm) the diameter using small calipers, and the circumference using a measuring tape.



**Figure 28**. Metatarsus measurements – measure diameter & circumference at the same point

## 3. FAT

## 3.1 Direct measure of backfat (carcass)

Equipment list:

- sharp knife
- measuring tape or ruler

If the animal has fat, with a sharp knife, make a 45° cut directly through the back fat layer on the rump near the base of the tail. The cut should be approximately 15-18 cm (6-7 inches) long across the rump, from the base of the tail. Spread the cut carefully so you don't distort (stretch or compress) the fat layer. At the deepest point along the cut, measure the depth of fat (to the nearest millimeter) from the connective tissue above the muscle to the top of the fat against the skin. Depth of back fat may also be measured after the animal has been skinned, if care is taken to remove the hide without any fat (Figure 29). Note if fat was still soft, or cool and hard. Remove back fat from ½ the animal, weigh, multiply by 2 and record.



**Figure 29**. Back fat depth. Left: knife showing cut angle from base of tail on skinned animal. Right: measuring depth of back fat.

## 3.2 Hunter assessment of fatness

Ask hunters to assess fatness of the animals based on amount of fat around the gut and kidneys, depth of back fat, and appearance of bone marrow. Fatness recorded as:

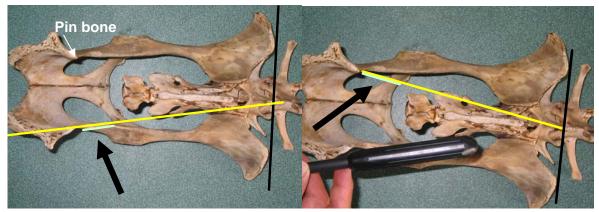
- skinny (no back fat, little or no gut or kidney fat, bone marrow red and runny)
- not bad (not too skinny) (little back fat, some gut or kidney fat, bone marrow pink and greasy)
- fat (nice layer back fat and plenty of gut or kidney fat, bone marrow a bit greasy and slightly pink)
- Very fat (thick layer back fat all the way up the back and fat everywhere in gut and around kidney, bone marrow solid and cream colored)

These categories corresponded to body condition scores based on:

- back fat
- gut fat
- kidney fat
- appearance of bone marrow

## 3.3 Ultrasound measures of back fat (live capture)

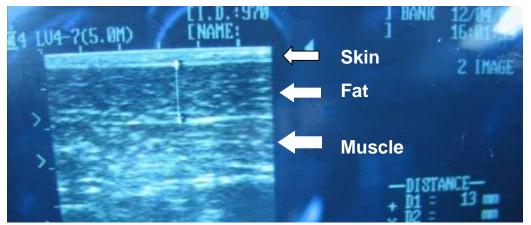
Ultrasound measures, along with back fat thickness of harvested animals, will be used to validate fatness level determined by palpation. Back fat should be measured at the place of maximum subcutaneous fat depth on the rump. Position the animal in sternal recumbency (lying on chest and belly). Place the transducer probe of the portable ultrasound along a line between the spine, at its closest point to the hipbone, and the pin bone. Measure the maximum fat thickness (immediately adjacent to the cranial process of the pin bone – see Figure 30) to the nearest 0.1 cm with the electronic calipers of the ultrasound equipment.



Photos by D.C. Heard

**Figure 30.** Dorsal view - location of ultrasound in relation to hip girdle (posterior to left; anterior to right).

In Figure 30, the point of maximum fat depth is marked by the green area along the yellow line where the thick black arrow is pointing. The yellow line extends from the (pin bone) (cranial process of the tuber ischium) to a point where a (black) line between the hip bones (tuber coxae) crosses the spine. If fat is present, it should be visible on the ultrasound image (Figure 31).



**Figure 31**. Maximum rump fat thickness determined by ultrasonography for caribou (photo, D.C. Heard).

## 3.4 Palpation for live animals or recently harvested animals

Equipment list:

bare (gloved) hands and eyes

Palpation is used on live animals to determine overall fatness. (Note that Ultrasound and palpation are different. Palpation uses scores, but has not been calibrated to back fat. Still it is a useful indicator of back fat and overall fatness.)

For harvested animals, palpation must occur within 30 minutes from the time of death (at which time the carcass starts to stiffen). Using the chart below, assign palpation scores to shoulder, ribs and hips-spine. The three points won't necessarily be the same. Mark score once per body area (i.e., shoulder / ribs / hips-spine). Scores may be broken down to halves (e.g. 2.5, 3.5)

#### SHOULDER

Score	Description
1	V-shaped, very bony. Hollows behind scapula, immediately behind spine.
2	Less bony. Hollow still present.
3	U-shaped withers. Hollow filled.
4	Very broad, U-shaped. Hard to feel edges of the bone.

#### RIBS

Score	Description
1	Deep grooves between ribs, even immediately behind shoulder
2	Ribs fairly well covered immediately behind shoulder.
3	Can still feel ribs, but grooves are not too deep.
4	Ribs nearly flush with tissue between them.

#### **HIPS / SPINE**

Score	Description
1	Hip bones very distinct (no back fat). Spine very distinct.
2	Some padding over hips. Spine very distinct.
3	Hips fairly well padded. Spine partly covered along each side.
4	Hips well padded. Spine is flush with or nearly covered with fat.

Total score (sum of shoulder, rib, hip/spine): \_\_\_\_\_

## 3.5 Metatarsus marrow fat

Equipment list:

- tools for removing bone marrow
- oven proof containers
- heat and water proof labels and markers
- drying oven (85°C)
- electronic scales (capable of measuring to the nearest 0.1 gram)

#### Record marrow fat characteristics. Categories are:

- o good: light colored and/or firm
- poor: pink and/or semi-solid
- o very poor: red and/or runny

#### Direct measure - lab processing

Storing and thawing bones. Metatarsus bones need to be kept in airtight storage containers in the freezer. The marrow fat estimate is based on marrow water content, so the bones should not be allowed to dry out. Marrow fat extraction should be done as soon as possible and certainly storage time shouldn't exceed a few months. The metatarsus must be thawed but the marrow extraction may be easier if the bone is still chilly. (Bones may be removed from the freezer and left in a cool room to thaw overnight). The marrow of each bone will be oven-dried in its own individual container over several hours or days, so determine how many containers will fit into your oven at once to determine how many bones to thaw (and marrow samples to process) at one time.

Once the bone is thawed, record the weight, length, circumference, diameter, and number of *Besnoitia* cysts.

<u>Extract the marrow</u>. If the metatarsus has been frozen and partially thawed, it may be easier to break the bones in a vice before extracting the marrow. Good gloves help with keeping a grip on the bone and will protect your hands from the occasional bone sliver. Use a counter-mounted vice to crack the bone but try not to crush it since bone fragments will enter the marrow. If you start in the middle and begin a crack, you can often extend the crack to the ends of the bone by moving the bone along in the vice and cracking again. If the metatarsus is fresh, it may be easier (and faster) to saw the ends off the metatarsus, and extract the marrow from ends and middle (Figure 32).



Figure 32. Metatarsus with ends sawed for removing marrow

<u>Remove the marrow</u>. Once the bone is broken and the marrow exposed, remove as much marrow as possible. Probes, small lab spatulas or small, dull carving tools work well to pick the marrow out of the ends. Put the marrow into clean, waterproof and heatproof containers such as small beakers (50 ml or 100 ml) or disposable foil trays (Figure 33).

<u>Prepare the container and label.</u> Each container will have a slightly different weight so weigh each container before use. Label the container with the ID number (make sure the labels are waterproof and heat proof as well – wax pencils or paper labels with ink won't do). If you're not using metal tags with ID numbers etched into them (they're nice but expensive), try using scraps of Tyvek or waterproof paper marked with soft pencil or indelible felt pens. If you put the tag in the empty beaker before weighing the beaker, then you can leave the tag in for the later weights that you will determine. Record the weight of the container and tag to the nearest 0.1 gram.

<u>Weigh the wet marrow.</u> Place all the marrow from each metatarsus in one container. Weigh the container with tag, and "wet" marrow.

<u>Dry the marrow.</u> Dry the marrow in its container in an oven at about 85°Celsius. Check the weight at 24 hours and 48 hours. (Do the weighing before samples are fully cooled.) If weights are different, then put the container back in the oven for another 12 to 24 hours (for a total drying time of 72 hours or more if needed). When weights are consistent, drying is complete.



Figure 33. Bone marrow from metatarsus. Left: fresh. Right: dried for 48 hours.

## 3.6 Kidney fat

Equipment list:

- clean, labeled plastic bags
- scale
- knife

#### Collection

<u>Always measure the LEFT kidney.</u> The left kidney is the one that is loose in the gut cavity on the left side of the caribou's body. (The right kidney is high against the back on the right side of the caribou and is attached to the liver - do NOT measure the right kidney.) The LEFT kidney should be collected with all attached fat: cut along the tissue connecting it to the body close to the spine (Figure 34). Freeze the kidney in a clearly labeled plastic bag for transport and storage. This kidney may be used for assessing pathogens as well.

**NOTE:** If both pathogens and contaminants are being assessed, then both kidneys should be collected, but use the **LEFT** kidney to assess fat before processing the kidney for other analysis.

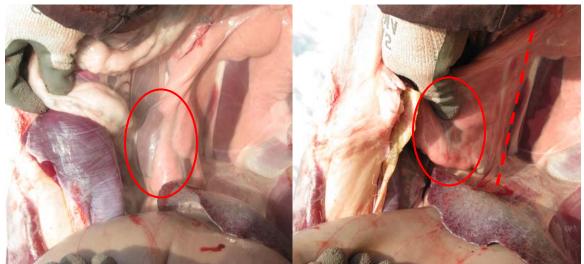


Figure 34. Kidney in body cavity

Cut off connected fat where it joins the body/abdominal wall, as shown by the dashed red line in Figure 34. Record if kidney:

- has no fat
- has very little fat
- is visible through the fat
- is completely covered and not visible through the fat

## Lab processing

Try to measure weights prior to freezing. If this is not possible, cut the fat while still frozen as in the following instructions, and weigh the sample while still frozen, as samples tend to loose water after they have been frozen and thawed. a. Weigh (to the nearest mg), the LEFT kidney with all the fat attached (Figure 35).

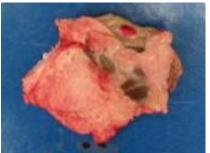
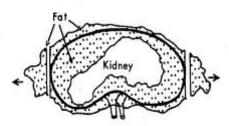


Figure 35. LEFT kidney with all fat

b. If there is fat at each end of the kidney (Figure 36), cut the fat perpendicular to the mid-line of the kidney and weigh the kidney with the remaining attached fat (Riney Index, Riney 1955).



**Figure 36**. LEFT kidney fat sample collection and cut lines for Riney kidney fat index calculation.

It is important to do these cuts properly in the field because kidneys must be frozen for storage and they are hard to "reconstruct" and cut properly after they are thawed. (After these cuts are made, the kidney *may* be frozen in a labeled plastic bag for transport and storage.)

- c. Remove all the remaining fat and connective tissue from the LEFT kidney, and weigh this fat along with the fat from "b".
- d. Weigh the kidney (to the nearest mg).

Calculate kidney fat mass by subtracting kidney weight from total weight of kidney with all fat attached (a-d). Record.

Riney kidney fat index is calculated as: kidney fat weight after perpendicular cut fat has been removed (b-d)/ left kidney weight (d). Record.

(RINEY, T. 1955. Evaluating condition of free-ranging red deer (*Cervus elaphus*) with special reference to New Zealand. New Zealand Journal of Science, 36: 429-463.)

# 4. INDIVIDUAL HEALTH

# 4.1 Blood sampling

Equipment list:

- filter strips
- gloves (one pair per animal)
- pencil or indelible pen for labelling
- labeled envelopes
- vacutainers or monovettes
- syringes
- warm packs
- 1 ml cryovials
- pipettes
- centrifuge

#### Collection on filter paper (blood sampling strips)

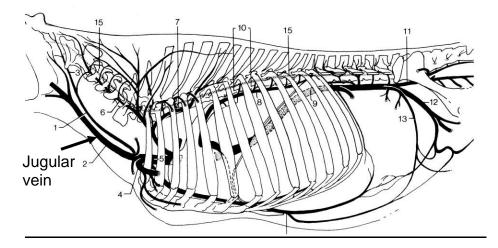
The use of filter paper will be validated over the next couple of years for testing pathogens, hormones, vitamins, minerals, and enzymes that can be detected in the blood. This technique has been used in human medicine for 50 years and is an economical and practical field collection technique for wildlife. Each strip holds 0.1 ml of whole blood (Figure 37), and is designed for use in a single analysis. Hunters dip the strips in blood from the heart or femoral vein (i.e., big vein on inside of hind leg) or neck vein or artery. Blood is usually collected onto 15 (3 sets of 5) strips per animal. This provides enough blood for immediate testing as well as banking for future analyses. Blood soaked filter paper (which are labeled with animal ID) should be first dried thoroughly and then frozen at -20° C. If drying is not possible immediately, then place the blood soaked filter papers in labeled paper envelopes for transport. Within 24 hours, the filters should be dried and then frozen in labeled bags.



**Figure 37**. A set of Nobuto blood sampling strips before and after blood sampling (blood strips on the right have been dried).

#### Collection by vacutainer / monovette

Blood collections from live caribou during capture-collaring must be done by a veterinarian or a trained field worker. Field staff MUST be trained (usually by the wildlife vet in your jurisdiction) and fully capable of doing collections prior to field season. Collect at least 20 ml of blood for serum and 10 ml of whole blood from femoral vein (the leg) or the jugular vein (Figure 38). (Always record which source was used.) From live animals, blood can be collected using vacutainers or something similar. You can also put a syringe needle (e.g. Precision Glide by Becton Dickinson, Franklin Lakes, NJ) on the vacutainer for drawing a blood sample intravenously from live animals). Tubes will have to be kept warm in an inner parka pocket or insulated container with warm packs if the outside temperature is at or below freezing.



**Figure 38**. Jugular vein (from: Dyce KM, Sack WO, Wensing CJG (1987) *Textbook of Veterinary Anatomy*, WB Saunders Company, Philadelphia-London-Toronto)

#### Blood samples from harvested animals

Luer Monovettes (Sarstedt Inc. Newton, NC) are excellent for collecting blood samples from harvested animals. (You can draw up a sample from a pool of blood in the body cavity but you must be quick and careful because blood coagulates quickly and contaminants (rumen fill and other fluids) ruin the sample. It works best to cut either the carotid or the hepatic artery and quickly draw up your sample from the fresh blood flowing from the artery. Alternatively you can use the femoral vein when the hind leg is cut off.

Keep monovette or vacutainer cool and prevent from freezing (in an inner parka pocket or insulated container with warm packs) until you are able to centrifuge the samples.

## **Centrifuging**

Allow samples to sit for at least an hour to clot before centrifuging. (If kept cool, blood samples may be kept for 12 hours before centrifuging.) Centrifuge the

vacutainers or monovettes for minimum of 10 minutes at least 3000 revolutions per minute (rpm) and transfer serum by pipette into 1-ml vials (e.g. Cryovails, Simport Plastics, Beloell, Quebec). You can use larger capacity vials but most tests require a small amount of serum - about 1 ml. If you need to send samples to different labs for different tests, you will end up having to thaw and divide larger samples into smaller vials. Use a fresh pipette for each caribou sampled to prevent contamination.

If live caribou are sampled after the rut, reserve 0.5 ml of serum from females for pregnancy (progesterone) testing. Serum is also used to detect the presence of antibodies for an array of pathogens.

Once you have processed blood samples, freeze the cryovials, monovettes and whole samples as soon as possible.

# 4.2 Disease, parasites and contaminants

Equipment list:

- labeled Whirl-Pak or Ziploc bags
- clean latex gloves
- scalpels

Need 20 samples per season.

Infectious diseases, contaminants, and chronic stress along with overall body condition influence the resilience of *Rangifer* to environmental change. Measures of infectious disease, contaminants, and stress are an essential component of every reference herd assessment and monitoring program. See Appendix 2 for more detail on contaminants sampling and analysis.

#### Harvested caribou:

The following samples allow for a wide variety of analyses of body condition indicators and historic growth rates in relation to contaminants, trace vitamins and minerals, infectious diseases and parasites. The sampling protocol is not too time-consuming or prohibitive for trained field collectors, and can be adapted/ modified annually as new needs are identified.

*Contaminants Collections*: 500g of both liver and muscle and one entire kidney should be collected from each animal. Each sample should be stored separately in a clean plastic bag (suggest Whirl-Pak or Ziploc) labeled and frozen as soon as possible. If possible, clean latex gloves and clean scalpels should be used to take the tissue samples, to avoid cross contamination between samples.

Laboratory processing and analysis: If samples were not collected with clean gloves and scalpels, a clean subsample of the liver and muscle should be taken in the laboratory (i.e. cut away potentially contaminated external surfaces of the tissue sample). If the kidney was not collected "cleanly", then the kidney capsule should be removed before the sample is processed. Because kidney tissue is

not homogeneous, the contaminants researcher should choose whether to analyze the kidney cortex, medulla or the kidney as a whole (in this case the kidney must be homogenized before being analyzed). Commercial or university labs will analyze the tissue samples for a suite of metals using inductively coupled plasma with mass spectroscopy (ICP-MS). This is the optimal method of analysis because the low detection limits enable quantification of metals that may be present in the tissues in very low quantities.

# BESNOITIA:

Equipment list:

- 100ml bottle of formalin
- red thread

Examine the bulbar and palpebral conjunctiva of the eyes for *Besnoitia* (Figure 39).



Figure 39. Besnoitia cysts look like grains of salt on the whites of the eyes.

Also examine skin on the rostrum, metatarsus, inner thigh and scrotum/udder for signs of *Besnoitia*. Ideally, the lower leg (metatarsus) will be collected with skin on such that it can be examined in the lab for signs of *Besnoitia*. If the metatarsus is not being collected with skin on, then check for *Besnoitia* by visual examination and by rubbing your naked index finger along the bone surface of the metatarsus, noting any roughness. Do the same for the rostrum by removing skin above the rostrum bone and rubbing your naked index finger along the bone surface of surface of the rostrum bone and rubbing your naked index finger along the bone surface of surface of the rostrum bone and rubbing your naked index finger along the bone surface of surface of the rostrum bone and rubbing your naked index finger along the bone surface of surface of the rostrum bone and rubbing your naked index finger along the bone surface of the rostrum bone and rubbing your naked index finger along the bone surface of the rostrum bone and rubbing your naked index finger along the bone surface of the rostrum bone and rubbing your naked index finger along the bone surface of the rostrum bone and rubbing your naked index finger along the bone surface of the rostrum bone and rubbing your naked index finger along the bone surface of the rostrum bone and rubbing your naked index finger along the bone surface of the rostrum horizon and you provide the rostrum bone and rubbing y

If a full collect of skin and tissue samples is of interest, and there are resources (and a qualified lab) available to analyze the samples, then the following may be collected into one (1) 100ml bottle of formalin. These samples are for histology, therefore do not crush the tissue, and keep the sample sizes small.

Tissue thickness should be  $\leq$  1 cm for good formalin penetration. Formalin can be discarded after 4 days and samples can be shipped 'dry' with a formalin-wet cloth in the bottle.

- SKIN Metatarsus Piece of metatarsus skin. Cut 1x2 cm skin from front mid-third of left metatarsus and look for *Besnoitia* under the surface. Attach red thread (to ID as metatarsus skin) before placing it in formalin.
- SKIN Nose Piece of nose (skin and cartilage). Pinch the full thickness of skin on top of the nose where the nasal rostrum bone ends and the cartilage starts. On the transverse plane (across the nose), cut deep enough (about 1 cm) to get a 1cm<sup>3</sup> chunk of both skin and nasal-turbinal cartilage. (It will look like an "M" in cross section)(Figure 40). If it is not possible to collect the cartilage, just collect a piece of the skin.



**Figure 40**. *Besnoitia*: How / where to cut for nasal skin piece, note the "M" of nasal turbinal/cartilage, get the "M" along with your skin sample.

- **SKIN Thigh** Piece of inner thigh. Cut 2 cm<sup>2</sup> of skin about 10 cm distally down the leg from the groin (towards hoof).
- SKIN Scrotum (male)/ Udder (female) If the scrotal bag seems normal, cut 1x2 cm piece of skin from it. If the scrotal bag does not seem normal (i.e. without hair, thick, or ulcerated) (Figure 41), cut a piece ½ on normal tissue and ½ on the ulcer/scab. For the udder you can cut 1x2 cm piece halfway on the skin with hair and halfway on the less hairy part. (This separation is easier to see than on the scrotal bag.)



Figure 41. Ulcerated scrotum

## **ORGAN and MUSCLE PARASITES:**

Equipment list

- scales
- 70% alcohol jars
- Ziploc bags

Estimate warbles, recent dermal scars, throat Botflies and sinus Tapeworms using the following quantities classifications: [0] [0-20] [20-100] [100-1,000] [>1,000]

If organ weights are of interest, weigh separately the heart, lungs with trachea, and liver to the nearest mg.

## Heart

Examine for *Taenia* cysts. Cut through the heart by sagittal section. Count and record number of cysts.

#### Lungs

Examine lungs for hardened areas that look like eyes (Figure 42). These could be hydatid cysts (*Echinococcus*), and filled with larval tapeworms. Examine lungs for *Dictyocaulus* nematodes (lungworm), which are 1-3 cm long, white, fairly thick/robust and obvious to the eye (Figure 43). Place lungs dorsal side up Using scissors or a knife, start cutting from the trachea, down the bronchi and bronchioles on one lung until the scissors can go no further. The worms will likely be present in the furthest reaches of the lung. Examine and record the number of worms found. Repeat on the other side. Gently scoop both male (small) and female (large) worms into jars of 70% Ethanol. Do NOT squeeze worms with pincers or wash in water as they will burst.



Figure 42. Hydatid cysts in the lungs



Figure 43. Dictyocaulus nematodes (lungworm)

## Liver

Equipment list:

- Ziploc bags
- clean lab gloves

Examine for parasites, liver flukes. Slice through the liver at regular intervals as you would slice through a loaf of bread. Freeze 500 g for contaminants analysis.

#### Muscle

Examine muscle tissue for tapeworm cysts (Figure 44). Record frequency.

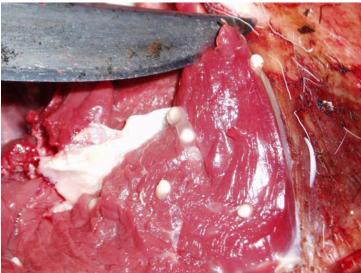


Figure 44. Tapeworm cysts in muscle

## Skin

Collect (in formalin 1 warble larva for DNA. Collect, weigh and freeze 10 warble larvae for archive. Count warble larvae on the back (use click counter).

#### Throat

Collect (in alcohol) 1 Botfly larva for DNA. Collect, weigh and freeze 10 Botfly larvae for archive. Look in nasal sinuses for Bots & Sinus Tapeworm. Count using classifications below.

#### Other

Note and collect any abnormalities. Specifically examine joints and testicles for *Brucella* and hair for ectoparasites.

## Disease Overview Tissue Collection (freeze all samples)

<u>These collections</u> will involve extensive post mortems and organ/tissue collections with quantification of parasites and pathogens in skin, muscles, lungs, gastrointestinal tracts and extensive testing of blood and organs for pathogens, trace vitamins and minerals etc. The extent of collection will depend on the purpose of the study.

#### REFER TO DISEASE TABLE: APPENDIX 3

Examine various organs for specific visible abnormalities and describe any additional abnormalities. Collect organs and tissues with abnormalities.

If gut parasites are of interest, collect and freeze the following:

- 1) **LUNGS** (for lungworm)
- 2) **ABOMASUMS** whole and tied off at the junction of the Omasum and Small intestine (for gastrointestinal parasites)
- 3) **SMALL INTESTINE** (3 meters intact, tied off) (for gastrointestinal parasites)

If brain disease (e.g. chronic wasting disease) is of interest, collect about 10 g tissue of the foramen magnum (Figure 45) and deposit in a labeled bag or bottle, and freeze.

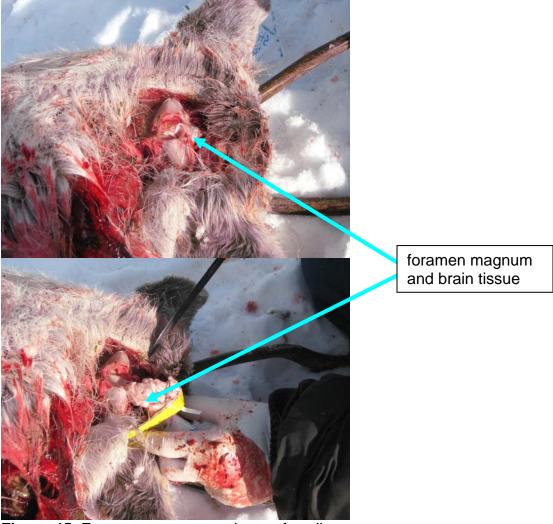


Figure 45. Foramen magnum at base of scull.

# 4.3 Diet from Fecal Samples (and option of pathogen analysis)

Equipment list:

- Ziploc bags
- clean lab gloves

Fecal samples can be collected from the lower gut once the animal has been eviscerated. Some can be used for diet analysis (see section 7 below). The rest can be used to test for the presence of helminthes, protozoa, viruses and bacteria. Feces, that are to be analyzed for parasites within one week, need to be kept cool (4°C), but not frozen. Alternatively, they should be frozen and analyzed within 4 months. NOTE: even one freeze-thaw cycle will reduce recovery of fecal parasites, it is therefore imperative that this is considered if comparing across time or populations. Feces can also be tested for bacteria, viruses, and parasites through culture and molecular techniques. Unless these are to be processed immediately they should be frozen as soon as possible. Fecal corticosteroid levels provide an indication of stress.

If possible, collect a minimum of 20 pellets per collection, using clean lab gloves on your hands, and placing the pellets directly into labeled Ziplock or Whirl-Pak plastic bags

# 5. MATERNAL STATUS

Equipment list:

- Nalgene bottles
- small vials with alcohol

Report milk in the udder as present or absent.

If milk is present, record characteristics:

- clear fluid weaning has just occurred
- milky fluid dilute milk (<20% dry matter indicating extended winter lactation)
- thick (like toothpaste) concentrated milk (> 25% dry matter)

#### Milk samples

Express milk from each quarter of the mammary gland and collect directly into a clean (new) nalgene bottle (one sample per cow). Freeze and store at <-10°C for analysis of Dry Matter content. (May also be analyzed for protein, fat and ash).

#### Uteri and ovaries

If reproductive history is of interest, collect ovaries for analysis of reproductive history. Store ovaries in a small vial with 97% alcohol for retrospective analysis of reproductive history. Note: this requires specialized skills, so resources must be available for a qualified lab to do the analyses.

#### <u>Fetus</u>

If a fetus is present, record size as described in section 2.3 "Body size - fetus"

# 6. PROTEIN BALANCE

Equipment list:

- surgical gloves for sampling and handling blood
- labels
- indelible marking pens or pencils
- plastic bottles for urine, snow urine, rumen contents
- large pail and large mixing spoon for mixing rumen contents
- Whirl-Pak bags for fecals and muscle tissue samples (or use bottles)
- scales for weighing tissue and rumen content samples
- freezer
- blood sampling equipment (monovetes)
- centrifuge
- pipettes
- cryotubes
- shipping units for frozen samples

#### Muscle Mass:

Weigh individual muscles, gastrocnemius and/or peroneus (to nearest 1/10 gm), to track seasonal changes in lean body mass relative to whole body mass (Refer to Figures 19 through 22. If using the meat of the animal is priority, then take only the peroneus muscle.

#### Nitrogen isotopes to determine protein partitioning:

Three samples that are representative of the animal's body, its diet, and the proportion of <sup>15</sup>N urea derived from the body are needed for this determination. This can be achieved in one of two ways:

#### Live capture

Collect blood sample: Separate whole blood by centrifuging (10min @ 3000rpm) into serum (for <sup>15</sup>N urea) and clot (indicator of body). Transfer the 1-3 mL serum and 1-3mL of the clot into separate plastic tubes (cryotubes) and freeze. Use a clean pipette for each sample.

Collect fecal pellets: These can be collected into Whirl-Pak plastic bags or plastic bottles and kept frozen.

## Field collection from harvested caribou:

Collect

- 1) blood serum *or* urine from the bladder (for <sup>15</sup>N urea),
- 2) blood clot or muscle tissue (indicator of the body), and
- 3) fecal sample (indicator of the diet; rumen content samples may be validated in the future for this purpose).

<u>Fetal blood sample</u> – hang fetus upside down and slit neck at throat as bend head back, have blood collection tubes immediately ready together any blood that drips out, squeeze / pump the fetus to press blood out.

Keep samples frozen. (Long term storage -20°C.)

Separate whole blood by centrifuging (10min @ 3000rpm) into serum and clot. Transfer the 1-3mL serum and 1-3mL of the clot into separate plastic tubes (cryotubes) and freeze. Use a clean pipette for each sample.

<u>Collect feces</u>. Collect 20g (usually about 20 pellets) Collect muscle tissue. Using a clean scalpel, collect

- Fetal tissue 20g of muscle from the rump
- Cow muscle tissue 20g from the flank

<u>Collect rumen contents</u>. If possible, pour ventral rumen contents into a large container and stir to obtain an even mix then collect 30g into a Nalgene bottle. Cool immediately to stop the fermentation.

<u>Collect urine sample</u>. Get 1-3ml from the bladder (dead animals often don't have urine in the bladder) or, if possible, 20 g of snow urine if you know for sure the urine came from the animal in question.

# 7. DIET from FECAL SAMPLES

Fecal samples from harvested animals may be collected from the lower gut, once the animal has been eviscerated. Fecal samples gathered at an area used by a group of caribou (e.g., fresh fecal pellets found on the ground or snow after a group of caribou has left the area), should be composite samples. Collect at least 10 pellets from each pile of fecal pellets, bag them separately, and create a composite sample for that site. (i.e., if you have collected fecal pellets from 13 separate piles, your composite sample will consist of one pellet from each of the 13 sample bags). Reserve the remainder for archive. Preserve all in salt or by freezing. Be meticulous with labeling. **NOTE** – If the sample is going to be used for other purposes (for example, analysis of protein partitioning), preserve by freezing rather than using salt.

#### Lab analysis:

Contact the Wildlife Habitat /Nutrition Lab, Washington State University for price estimates - see Appendix 1 for contact information.

# 8. GENETIC TYPING

Contact the genetic typing lab that you will be working with in advance of starting your collection to determine details regarding sample processing and preservation, and preferred sample sizes. Generally, hair with follicle and antler pedicel samples will have to be kept clean (uncontaminated by other genetic material) and frozen.

Estimated Sample size: 20 per herd

Collections may include:

- blood
- dry blood on filter paper
- hair with follicle
- antler pedicel
- fresh muscle tissue frozen

# **APPENDIX 1. Contact Information**

#### Morphology:

Dorothy Cooley Regional Biologist Department of Environment Government of Yukon Box 600 Dawson City, Yukon Y0B 1G0 Ph: 867-993-6461 Email: <u>Dorothy.Cooley@gov.yk.ca</u>

Dr. Robert G. White Scientist Emeritus University of Alaska email: <u>ffrgw@uaf.edu</u>

Jan Adamczewski Ungulate Biologist Department of Environment and Natural Resources P.O. Box 1320 Government of the NWT Yellowknife, NT X1A 2L9 Ph: 867-873-7763 email: Jan Adamczewski@gov.nt.ca

#### Pathogens:

Dr. Susan Kutz Ingenuity New Faculty Associate Professor Faculty of Veterinary Medicine University of Calgary 3330 Hospital Drive NW Calgary, Alberta, Canada, T2N 4N1 Ph: 403-210-3824 fax: 403 210-3939 email: skutz@ucalgary.ca

#### Contaminants:

Mary Gamberg Gamberg Consulting Box 10460 Whitehorse, Yukon Canada Y1A 7A1 Ph:867-668-7023 Fax:867-668-7024 email: mary.gamberg@northwestel.net

#### **Genetic Typing**:

Kris Hundertmark Assistant Professor of Wildlife Ecology Institute of Arctic Biology University of Alaska Fairbanks 412 Irving I Fairbanks, AK, USA 99775 Ph: 907-474-7159 ffkh@uaf.edu

#### Protein partitioning:

Perry Barboza Associate Professor Department of Biology & Wildlife Institute of Arctic Biology University of Alaska Fairbanks PO Box 757000 Fairbanks, AK, USA 99775-7000 Ph: 907-474-7142 email: <u>ffpsb@uaf.edu</u>

Dr. Katherine Parker Professor Natural Resources and Environmental Studies University of Northern British Columbia 3333 University Way Prince George, BC V2N 4Z9 Ph: 250-960-5812 email: parker@unbc.ca

#### Lab analysis of fecal plant fragments:

Contact this lab for price estimates: Bruce Davitt Supervisor, Wildlife Habitat /Nutrition Lab Dept. Natural Resource Sciences Plant Biosciences 1-Johnson Hall Addition, Room 136 Washington State University Pullman, WA USA 99164-6410 Ph: 509-335-2318 Fax: 509-335-7862

#### Cementum age:

Matson's Laboratory LLC US Post Office address: PO Box 308, Milltown MT 59851 USA Physical address (for UPS, FedEx, and other couriers: 8140 Flagler Road, Milltown MT 59851 USA email: gimatson@montana.com

Timing of collections	Number of samples required	Collection method	Transport and handling of sample	Sample Storage - temperature - preservative	List of variables analyzed	Location and cost of analysis (per sample )
Fall (preferred) or spring	20/herd	Whole kidney	Keep frozen	Store frozen in clean labeled Ziplock/Whirl- Pak –40C if possible	Ag,Al,As,B,Ba,Be, Bi,Ca,Cd,Co,Cr,Cs, Cu,Fe,Ga,Hg,K,La,Li Mg,Mn,Mo,Ni,Pb,Pd, Pt,Rb,Sb,Se,Sn,Sr Tl,U,V,Zn	Processing by Mary Gamberg \$40/sample set (ie. per animal) Analysis by NLET (Env Can, Burlington) \$120/sample
Fall (preferred) or spring	20/herd	500 g solid liver tissue	Keep frozen	Store frozen in clean labeled Ziplock/Whirl- Pak –40C if possible	Ag,Al,As,B,Ba,Be, Bi,Ca,Cd,Co,Cr,Cs, Cu,Fe,Ga,Hg,K,La,Li Mg,Mn,Mo,Ni,Pb,Pd, Pt,Rb,Sb,Se,Sn,Sr Tl,U,V,Zn	Processing by Mary Gamberg \$40/sample set (ie. per animal) Analysis by NLET (Env Can, Burlington) \$100/sample
Fall (preferred) or spring	20/herd	500 gm solid muscle tissue	Keep frozen	Store frozen in clean labeled Ziplock/Whirl- Pak –40C if possible	Ag,Al,As,B,Ba,Be, Bi,Ca,Cd,Co,Cr,Cs, Cu,Fe,Ga,Hg,K,La,Li Mg,Mn,Mo,Ni,Pb,Pd, Pt,Rb,Sb,Se,Sn,Sr Tl,U,V,Zn	Processing by Mary Gamberg \$40/sample set (ie. per animal) Analysis by NLET (Env Can, Burlington) \$100/sample
Fall (preferred) or spring	20/herd	Incisor bar	Keep frozen	Store frozen in labeled plastic bag	Age (year)	Aging by Wildlife Agency lab or commercial lab

# APPENDIX 2. Details of Contaminants Collection and Analysis

# APPENDIX 3. Known pathogens of North American caribou

From: Neimanis, A. and Kutz, S. The Sahtu Wildlife Health Monitor Program.

Pathogen	Subspecies affected	Sample(s) required for diagnosis	Reference
<i>Babesia</i> sp.	captive woodland caribou in Minnesota and Oklahoma	whole blood	(Holman, Petrini, Rhyan & Wagner, 1994); (Petrini, Holman, Rhyan, Jenkins & Wagner, 1995)
Besnoitia tarandi	woodland and barren-ground caribou; woodland caribou in SK	gross lesions, parasite	(Northwest Territories Resources Wildlife and Economic Development, 2002); (Wobeser, 1976)
Bluetongue virus	Alaskan caribou	serum (or blood on filter paper)	(Zarnke, 2000)
Bovicola tarandi (chewing lice)	northern caribou	gross lesions, parasite	(Durden, 2001)
Bovine adenovirus 3	caribou in QC	serum (or blood on filter paper)	(Elazhary, Frechette, Silim & Roy, 1981d)
Bovine respiratory syncytial virus	Northern Alaskan caribou	serum (or blood on filter paper)	(Zarnke, 2000)
Bovine viral diarrhea virus (or a cross- reacting virus)	caribou in QC, caribou in Alaska	serum (or blood on filter paper)	(Elazhary, Frechette, Silim & Roy, 1981c); (Elazhary, Roy & Frechette, 1979b); (Zarnke, 1983)
<i>Brucella suis</i> biovar 4	woodland caribou in Nahanni National Park , barren-ground caribou in NWT	serum (or blood on filter paper), gross lesions	(Northwest Territories Resources Wildlife and Economic Development, 2002); (Tessaro & Forbes, 1986)
Bunyamwera virus (species unspecified)	captive caribou, Wisconsin	serum (or blood on filter paper)	(Hoff, Spalatin, Trainer & Hanson, 1970)
<i>Cephenemyia trompe</i> (nose bot)	barren-ground caribou	gross lesions, parasite	(Northwest Territories Resources Wildlife and Economic Development, 2002)
Contagious ecthyma	northern caribou, Alaskan caribou	Gross lesions, serum (or blood on filter paper)	(Northwest Territories Resources Wildlife and Economic Development, 2002); (Zarnke, Dieterich, Nieland & Ranglack, 1983); (Zarnke, 1983)
Coronavirus	caribou in QC	serum (or blood on filter paper)	(Elazhary, Frechette, Silim & Roy, 1981b)

Pathogen	Subspecies affected	Sample(s) required for diagnosis	Reference
Cryptosporidium sp.	N. Alaskan caribou	feces	(Siefker, Rickard, Pharr, Simmons & O'Hara, 2002)
<i>Dermacentor albipictus</i> (winter tick)	woodland caribou in Alberta (Welch)	gross lesions, parasite	(Welch, Samuel & Wilke, 1990)
<i>Echinococcus granulosus</i> (Hydatid disease)	northern caribou	gross lesions, parasite	(Northwest Territories Resources Wildlife and Economic Development, 2002); (Rausch, 2003)
Elaphostrongylus rangiferi	caribou in NFLD	feces (Baermann)	(Lankester & Fong, 1998)
Epizootic hemorrhagic disease virus	Alaskan caribou	serum (or blood on filter paper)	(Zarnke, 2000)
Fascioloides magna (Giant liver fluke)	wild woodland caribou in QC	feces, parasite from liver	(Choquette, Gibson & Simard, 1971)
Fusobacterium necrophorum	caribou in the USA	gross lesions	(Rausch, 1953)
Giardia sp.	Caribou	feces	B. Elkin, pers. Comm
Hypoderma tarandi (warbles)	northern caribou	gross lesions, parasite	(Northwest Territories Resources Wildlife and Economic Development, 2002)
Infectious bovine rhinotracheitis virus (or a cross-reacting virus)	caribou in QC; woodland caribou in SK, Alaskan caribou	serum (or blood on filter paper)	(Elazhary, Frechette, Silim & Roy, 1981a); (Elazhary, Roy & Frechette, 1979a); (Jordan, Rettie & Tessaro, 2003); (Zarnke, 1983)
Leptospira interogans	woodland caribou in Yukon, Alaskan caribou	serum (or blood on filter paper)	B. Elkin, pers. comm.; (Zarnke, 1983)
Malignant catarrhal fever	Alaskan caribou	serum (or blood on filter paper)	(Zarnke, Li & Crawford, 2002)
Marshallagia marshalli	caribou in NWT and Nunavut	feces	(Hoberg, Kocan & Rickard, 2001)
Nematodirella spp. (alcidis, longissimespiculata)	caribou from Alaska and N. Canada	feces	(Hoberg et al., 2001)
Nematodirus spp. (filicollis, odocoilei, skrjabini, tarandi)	caribou from Alaska, NFLD, NWT, QC and BC	feces	(Hoberg et al., 2001)
Northway virus (arbovirus)	Alaskan wild caribou	serum (or blood on filter paper)	(Zarnke, Calisher & Kerschner, 1983); (Zarnke & Yuill, 1981)

Pathogen	Subspecies affected	Sample(s) required for diagnosis	Reference	
Ostertagia spp. (gruehneri, arctica, mossi).	caribou in Alaska and throughout Canada	feces	(Hoberg et al., 2001)	
Papillomas and fibropapillomas	barren-ground caribou, NWT	gross lesion	(Broughton, Miller & Choquette, 1972); (Northwest Territories Resources Wildlife and Economic Development, 2002)	
Parainfluenza virus 3	captive and wild caribou	serum (or blood on filter paper)	(Van Campen & Early, 2001)	
Parelaphostrongylus andersoni	woodland and barren-ground caribou in N. Canada and Alaska	feces (Baermann)	(Lankester, 2001)	
Parelaphostrongylus odocoilei	woodland caribou in AB	feces (Baermann)	(Gray & Samuel, 1986)	
Parelaphostrongylus tenuis	experimentally infected and introduced caribou	feces (but fatal, therefore should not be seen in healthy caribou)	(Lankester, 2001)	
Poxvirus	captive reindeer in the Toronto Zoo	gross lesions	(Robinson & Kerr, 2001)	
Rabies	northern caribou	serum (or blood on filter paper)	(Northwest Territories Resources Wildlife and Economic Development, 2002)	
Sarcocystis sp.	barren-ground caribou	gross lesions (only if severe), muscle, parasite	(Northwest Territories Resources Wildlife and Economic Development, 2002)	
Setaria labiatopapillosa	caribou in N. America	whole blood, parasites from carcass	(Becklund & Walker, 1969)	
Solenoptes tarandi (sucking lice)	northern caribou	parasites	(Durden, 2001)	
Taenia hydatigena (cystocercosis)	northern caribou	gross lesions, parasite	(Northwest Territories Resources Wildlife and Economic Development, 2002)	
<i>Taenia krabbei</i> (muscle cysts)	barren-ground and woodland caribou	gross lesions, parasite	(Northwest Territories Resources Wildlife and Economic Development, 2002)	

Pathogen	Subspecies affected	Sample(s) required for diagnosis	Reference
Teladorsagia spp. (boreoarcticus, circumcincta, trifurcata)	caribou from Alaska and northern Canada	feces	(Hoberg et al., 2001)
Toxoplasma gondii	barren-ground caribou, NWT and Nunavut	serum (or blood on filter paper)	(Kutz, Elkin, Panayi & Dubey, 2001)
Trichostrongylus axei	caribou from NFLD	feces	(Hoberg et al., 2001)
<i>Trypanosoma</i> sp.	wild woodland caribou, AB, barren- ground caribou in NWT	whole blood	(Lefebvre, Semalulu, Oatway & Nolan, 1997); (S. Kutz, pers. comm.)
West Nile Virus	captive reindeer	serum (or blood on filter paper)	(Palmer, Stoffregen, Rogers, Hamir, Richt, Pedersen & Waters, 2004)