Section 5.2.11 Livestock Sampling Methods: Cattle, Sheep, Goats, Camels, and Swine

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Objectives: To safely collect biological samples from livestock.

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The authors assert that animal capture and sampling should always occur in compliance with all applicable laws and regulations and should only be undertaken after securing all necessary permits and approvals, including ethical approvals.

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Table of Contents: Livestock Sampling Methods: Cattle, Sheep, Goats, Camels, and Swine

Section 5.2.11.a. Brief Overview of PPE

Minimum PPE Required for Livestock Sampling

The minimum PPE for livestock (including camels, cattle, sheep, goats, and swine) sampling includes:

1. Dedicated clothing
2. Nitrile (recommended) exam gloves
3. Safety glasses or other eye protection

(See the PREDICT ***Biosafety and PPE Guide (Section 4.1)*** for detailed instructions regarding PPE Use)

Standard disinfection procedures for equipment and clothing should be followed when moving between animal enclosures or properties.

Section 5.2.11.b. Livestock Handling and Welfare

Performance standards during handling include careful, considerate, respectful, calm, human interactions with animals in as positive a manner as is possible. Animals handled in a respectful manner will be calmer and easier to handle than animals handled in a rough or disrespectful manner. PREDICT field staff should be familiar with the correct techniques and the anatomy of each livestock species before attempting sampling procedures. At all times, observe animals for signs of excessive distress. If animals are unwell, stop all procedures, provide adequate support care, and release upon recovery.

While most veterinarians are familiar with handling livestock, we recommend that PREDICT staff visit the following guidelines as a refresher.
http://www.dardni.gov.uk/safe_cattle_handling_guidance.pdf

For more information on Animal Handling and Transport, see:
http://www.fass.org/docs/agguide3rd/Chapter05.pdf

For more information on welfare considerations for cattle handling, see:
http://www.animalwelfarestandards.net.au/files/2011/02/Cattle-Standards-and-Guidelines-for-Endorsement-May-0807141.pdf (Section 5, pages 13-16) and
Beef cows: **http://www.fass.org/docs/agguide3rd/Chapter06.pdf**
Dairy cows: **http://www.fass.org/docs/agguide3rd/Chapter07.pdf**

For more information on welfare considerations for sheep handling, see:
http://www.animalwelfarestandards.net.au/files/2011/02/Sheep-Standards-and-Guidelines-for-Endorsement-May-2014-080714.pdf (Section 5, pages 14 and 15) and
http://www.fass.org/docs/agguide3rd/Chapter10.pdf

For more information on welfare considerations for blood collection from cattle, see:
http://www.dpi.nsw.gov.au/agriculture/livestock/animal-welfare/general/livestock/sop/cattle/blood-collection

For more information on welfare considerations for swine handling, see:
http://www.fass.org/docs/agguide3rd/Chapter11.pdf

For more information on welfare considerations for camels handling, see:
http://www.publish.csiro.au/Books/download.cfm?ID=5204

Section 5.2.11.c. Sample Data Collection

Introductions and informed consent

Upon arriving to a household or farm, introduce yourselves (team members, purpose of the visit) to the acting head of household responsible for the livestock. Explain the purpose of the study, allow time for questions, and clarify any issues that may arise. If local regulations require it, obtain informed consent per project guidelines and protocols.

Animal Handling and Sampling Procedures

Note: For all food animals, manual restraint will be used. If drugs are used for sedation in a food animal, that animal will not be allowed to return the human food chain unless it is specifically labeled for use in that species and withdrawal periods are observed

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two nasal swabs** - one in 500 µL VTM and one in 500 µL Trizol
2. **Two fecal samples** - one with max of 500 µL/0.5cc feces in 500 µL VTM and one with max of 500 µL/0.5cc feces in 1 mL Trizol

   Or

   Two rectal swabs - one in 500 µL VTM and one in 500 µL Trizol
3. **Two whole blood samples** - one with max of 500 µL of whole blood in 500 µL VTM and one with max of 500 µL of whole blood in 500 µL Trizol
4. **Two serum samples** - 2 x 1.0 ml aliquots frozen without media
5. **Two urogenital swabs or urine samples** - one with max of 500 µL of urine in 500 µL VTM and one with max of 500 µL of urine in 500 µL Trizol

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e. within 24 hours) to cold chain such as in an emergency situation, then samples can be collected in 500 µL of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37 °C (i.e. ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80 °C for storage until analysis

Collecting Nasal Swabs

Using sterile, polyester-tipped swabs with a plastic shaft, rub the swab tip gently but thoroughly against the walls of the animal's nares, about 1-2" from the opening, saturating the swab with mucus. **Place 1 swab in a cryovial filled with 500 µl of VTM and the other swab into 500 µL of Trizol in another cryovial**. Mix each tube well. Store both cryovials in a liquid nitrogen dry shipper or dewar & transfer to -80˚C freezer when possible.

Bleeding Collection Techniques

1. Cattle

Blood can be collected from the jugular vein in cattle of all ages or from the tail (coccygeal) vein of older cattle.

A variety of collection devices may be used - vacutainers, bleeding tubes, syringe and needle. Restraint should ensure quick, easy and safe collection of the sample causing minimal distress. This may involve use of a bail, race, or crush for tail bleeding. For jugular bleeding the animal may require minimal restraint (e.g. halter) or may need to be restrained in a crush with head bail and the employment of a halter or nose grips. Use of nose grips should be avoided wherever possible.

Operators should use gloves and disinfect or replace them between animals to prevent the transmission of blood-borne diseases. Equipment such as vacutainer holders should also be cleaned between animals. An antiseptic must be applied to clean skin surface prior to venipuncture.

For a visual guide see the following online tutorials:

Cattle
https://www.youtube.com/watch?v=IuNbsTMrIuI (tail and jugular)
https://www.youtube.com/watch?v=ZEsHMwKFbKg (tail)
https://www.youtube.com/watch?v=812CskWCqGQ (jugular)

Procedure for Jugular Venipuncture Using Vacutainer Needle and Tubes:

1. Identify and georeference the study site and document the signalment of the animal on the data collection sheet.
2. Before sample collection, ensure that the animal is effectively and humanely restrained to avoid injury to the animal and/or study personnel.

3. Using the halter, position the animal's head so that it is slightly elevated and drawn to the side opposite the jugular vein to be sampled.
4. Disinfect venipuncture area with alcohol
5. Occlude the vein by applying digital pressure in the jugular groove located in the lower neck.
6. Place a vacutainer needle attached to a vacutainer holder into the distended jugular vein at a 45° angle cranial to the jugular groove.
7. Once needle is positioned in the vein, insert a vacutainer into the needle to collect the blood.
8. When the desired volume has been collected (5 ml minimum suggested) remove the occluding pressure from the vein.
9. Detach the tube from the needle and withdraw the needle from the jugular vein.
10. You can collect more than 1 tube by repeating steps 7 and 8.
11. Label the vacutainer tubes with the sample ID.

Procedure for Jugular and Coccygeal Venipuncture Using Syringe and Needle

Jugular bleeding

1. Restrain cow with the head elevated and the jugular groove exposed.
2. Raise the jugular vein by placing pressure at the base of the jugular groove.
3. Pass the needle through the skin and into the vein by a firm thrust directed at an angle of 20^{o} to the skin surface.
4. Withdraw the blood sample.

Tail Bleeding

1. Restraint should prevent the cow from moving away during the procedure.
2. Raise the tail vertically with one hand until it is horizontal with the ground.
3. Approximately 150 mm from the base of the tail, locate the groove lying in the ventral midline of the tail.
4. Midway along the body of a coccygeal vertebra, insert the needle perpendicularly to the surface of the skin to a depth of a few millimeters.
5. Withdraw blood sample.
6. Apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.

Once blood is collected, place the needle into a sharps container. Open red-top and purple top vacutainer tubes. Place approximately 2.5cc in each tube, then discard the syringe into a biohazard container. Invert each tube several times to mix.

2. Sheep/Goats

Blood should be collected from the jugular vein. The procedures for blood collection are identical to those described for cattle, with the exception of the amount of restraint needed and the possibility of shearing the bleeding area on the neck for easier viewing of the vein and minimizing the chance of introducing dirt or bacteria into the vein with the needle.

In sheep and goats, blood sampling can be done with assistance or alone. If you are not proficient at drawing blood alone, work with an assistant. The assistant should restrain the sheep/goat's body and turn the head to the side, at a 30-degree angle, by holding the animal under its jaw to allow for easy access to the jugular vein.

Restraining a sheep or goat without assistance is better for those who have become proficient at drawing blood. The handler should straddle the sheep/goat, place his or her knees behind the animal's shoulders, and back the sheep/goat into a corner or against a wall to help control their hindquarters. The sheep/goat's head should be turned opposite to the side of collection, once again at a 30-degree angle. Restraint of the head is accomplished by using the elbow and the upper arm to keep it held off to the side. This leaves both hands available for the blood collection.

The easiest way to locate the vein is to draw an imaginary line from the middle of the sheep/goat's eye down the side of the neck. The vein can be located by applying pressure with the thumb or fingers in the groove on either side of the trachea. The pressure will cause the vein to pop up and be easy to feel or see if the area has been shaved. Proceed as with cattle, using a vacutainer collection system or syringe and needle.

For a visual guide see the following online tutorials:

Sheep/goats (small ruminants) https://www.youtube.com/watch?v=47tlmqXX3eE

Blood sample processing and storage:

Whole Blood

- Collect whole blood into 1 lavender top tube containing EDTA, and allow another tube to clot for collection of serum.
- Add up to 500 µL of whole blood (from EDTA tube) directly into 2 vials, one containing 500 µL Trizol and one containing 500 µL VTM (= maximum final ratio of 1:1) and mix each vial well.

Serum

- After clotting is complete, use a plastic pipette to take 1 ml of serum and transfer into 2 cryovial tubes, 0.5 ml each.
- If a centrifuge is available, centrifuge samples for 15 minutes and then collect 1 ml serum and transfer into 2 cryovial tubes, 0.5 ml each.
- Label the cryovial tubes with the same label information used on vacutainer tube.
- You can harvest additional serum for serum bank as appropriate.
- Freeze all samples in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

3. Camels

Because of the risk of MERS CoV exposure, sample collectors should wear gloves, a respirator, and eye protection when handling camels.

Blood can be collected from the jugular vein in camels of all ages, though it is recommended that this be undertaken on animals while they are in sternal recumbency (kush position), well-restrained, or sedated. The lateral thoracic vein or caudal epigastric ("milk") vein may be used but should only be targeted in animals where physical or chemical restraint prevents kicking.

A vacutainer needle (18G or 19G) with purple top (EDTA) tubes and red-top (with serum clot activator) tubes may be used, or a 5cc syringe and 18G or 19G needle. Restraint should ensure quick, easy and safe collection of the sample causing minimal distress.

Equipment such as vacutainer holders should be cleaned between animals.

Procedure for Jugular Venipuncture Using Vacutainer Needle and Tubes

1. Identify and georeference the study site and document the signalment of the animal on the data collection sheet.
2. Before sample collection, ensure that the animal is effectively and humanely restrained to avoid injury to the animal and/or study personnel.
3. Using the halter, elevate the animal's head and draw it to the side opposite the jugular vein to be sampled.
4. Disinfect venipuncture area with alcohol
5. Occlude the vein by applying digital pressure in the jugular groove located in the lower neck. Alternatively, a rolled towel affixed with a rope over the withers can be applied at the same level to act as a temporary incomplete tourniquet.
6. Place a vacutainer needle, attached to a vacutainer holder, into the distended jugular vein at a 45° angle cranial to the jugular groove.
7. Once the needle is positioned in the vein, insert a vacutainer into the needle and collect the blood.
8. When the desired volume has been collected (5 ml minimum suggested), remove the occluding pressure.
9. Detach the tube from the needle.
10. Detach the needle from the jugular vein and apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.
11. If more than one tube of blood is required, repeat steps 7 through 9 with occluding pressure.
12. Label the vacutainer tubes with sample ID.

Note: If vacutainer needles are unavailable, a 5cc syringe and 18G or 19G needle can be used. Once blood is collected, place the needle into a sharps container. Open red-top and purple top vacutainer tubes. Place approximately 2.5cc in each tube, then discard the syringe into a biohazard container. Invert each tube several times to mix.

Whole blood can be aliquoted into cryotubes with VTM and Trizol using a pipette gun. Serum tubes can either be centrifuged (if available) or placed vertically in a cooler with ice bricks and allowed to stand undisturbed overnight (~12 hours) for clean serum separation. Serum can then be aliquoted into cryotubes.

Procedure for Jugular Venipuncture Using Syringe and Needle

Jugular bleeding

1. Restrain camel with the head elevated and the jugular groove exposed.
2. Disinfect venipuncture area with alcohol
3. Raise the jugular vein by pressure at the base of the jugular groove.
4. Pass the needle through the skin and into the vein by a firm thrust directed an angle of 20° to the skin surface.
5. Withdraw blood sample.
6. Apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.

Lateral Thoracic/Caudal Epigastric Vein Bleeding

1. Restraint should prevent the camel from moving away or kicking during the procedure.
2. Identify the lateral thoracic vein, caudal to the point of the elbow's olecranon process.
3. Pass the needle through the skin and into the vein by a firm thrust directed an angle of 20° to the skin surface.
4. Withdraw blood sample.
5. Apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.

4. Swine

All personnel handling or sampling pigs should wear appropriate PPE and practice appropriate biosafety practices to avoid spreading infection from one animal to another and from one herd, farm or property to another. This includes wearing dedicated clothing (e.g. coveralls and rubber boots) that can be removed and disinfected once work at a site has been completed. Recommended PPE includes nitrile gloves, a respirator and safety glasses.

Restraint: Manual restraint is recommended, without the use of anesthesia. Pigs to be sampled should be constrained to a separate pen, if possible. The use of a snout snare (see appendix) by the animal restrainer is recommended for pigs over 20 kg, but should only be used by experienced personnel and for short term restraint to avoid injury to the pig's snout. Pigs will be restrained for a maximum of three minutes and then released. If blood collection is unsuccessful, then the pig will be allowed to calm down for five minutes before a second attempt is made.

Blood can be collected from the external jugular vein, or the cranial vena cava, using a 1", 20G needle and a 5cc syringe. This technique requires the head to be restrained and elevated parallel to the ground, typically using a snout snare. In pigs weighing less than ~50 kg, blood can be collected further caudally (and more medially) in the jugular groove, nearer the manubrium from anastomose of internal and external jugular vein. For pigs weighing less than ~20 kg, a technician will manually restrain the pig on his lap, holding the forelegs in one hand, and the animal's head in the other. Then a max of 5.0 to 10 ml may be collected from the jugular vein. Venipuncture should only be performed by experienced personnel.

The marginal ear veins are the only veins that are easily visible on pigs of any size. Usually there are three prominent veins. The lateral or central vein is usually the largest of these. These veins may also be punctured for blood collection. An alternative venipuncture site is the caudal auricular ("marginal ear") vein, though this typically yields low (<1 mL) blood volumes. A smaller, 22G or 23G needle should be used for this vein.

See also http://oslovet.norecopa.no/teaching/pig/pigbleed/ for more details on blood collection from pigs.

Collecting Fecal Samples

Ensure the animal is properly restrained prior to sampling. Fresh fecal samples should be collected, preferably from the rectum. If freshly passed, feces can be collected off the ground. Only the top part of a freshly passed fecal pat should be collected using a disposable spoon or scooped up in a gloved hand, plastic bag or plastic vial.

For Collection from the Rectum in Cattle and Camels

- The operator places an obstetrical sleeve on one arm
- The arm is formed into a cone and the animal's tail held to one side with the opposite gloved hand.
- Gentle pressure is applied to the anal sphincter until penetration into the rectum is obtained.
- A fecal aliquot of sufficient size for the intended laboratory procedure is scooped with the sleeved hand and removed from the animal.
- The fecal sample is placed in a separate container or the obstetrical sleeve is inverted off the arm such that the fecal sample is trapped inside.

Small calves, sheep, goats, and swine: restrain manually. Gently pass a gloved, lubricated finger through the anus and massage the rectal wall to stimulate rectal evacuation. If feces are not produced, collect feces with finger.

Place two ~200 mg (pea size) samples of fresh feces into 2 vials, one containing 500 µL VTM (= maximum final ratio of 1:1) and one containing 1 mL Trizol (= maximum final ratio of 1:2). Homogenize by shaking. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.

If feces are not available, collect 2 rectal swabs- 1 in VTM and 1 in Trizol: Gently insert one sterile swab tip at a time into the animal's rectum. [Note: DO NOT USE TRIZOL AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE.] Place 1 swab in a cryovial filled with 500 µL of VTM. Place the other swab into a tube with 500 µL of Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80˚C freezer when possible.

Collecting Urine/Urogenital Swabs

Many animals will urinate as a fear reaction while they are handled. Urine can be collected free catch in plastic vials. Add up to 500 µL of urine directly into 2 vials, one containing 500 µL VTM and one containing 500 µL Trizol (= maximum final ratio of 1:1) and mix each tube well. Store in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.

If urine is not available, collect 2 urogenital swabs: 1 in VTM and 1 in Trizol. Place 1 swab in a cryovial filled with 500 µL of VTM. Place the other swab into a tube with 500 µL of Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80˚C freezer when possible.

Section 5.2.11.d. Sample Collection from Dead or Euthanized Livestock

PREDICT's primary approach to sample collection in livestock is to collect specimens from living animals. In the event that an animal has died of natural causes or been euthanized due to humane or veterinary care reasons, the guidelines below for necropsy sampling may be followed. If bodies are relatively whole and fairly fresh, then sample as described above. The *American Veterinary Medical Association guidelines (Section 8.5.2.)* in the PREDICT Operating Procedures ebook provides information on animal euthanasia that may be useful to PREDICT veterinarians called upon to euthanize an animal.

As discussed throughout this protocol, all animals should be considered potentially infectious for a wide variety of dangerous pathogens, and dead animals in particular should be sampled only following all safety measures, including proper PPE use, proper work station decontamination, and proper carcass disposal, as outlined here and in other PREDICT documents.

Though not required for PREDICT sampling, thorough necropsy procedures can be very beneficial and relevant for some animals (e.g., suspicious deaths). Time and skill permitting, when full necropsies are performed, following any Association of Zoos and Aquariums/AZA (or similar) necropsy protocol is recommended and most can be adjusted for application to livestock species. Necropsy protocols are also addressed in the Non-Human Primate Sampling protocol, Appendix V.: AAZV's Occupational Primate Disease Safety Guidelines for Zoological Institutions: Standardized Necropsy Report for Non-Human Primates Work Sheet (ebook Section 5.2.6j.); most of the information and worksheets in this document can be utilized for sampling of

livestock. (*Note that properly following extensive necropsy procedures and collecting and measuring all samples can require 4-6 hours for a single animal.*)

Duplicate blood samples are to be collected from each animal; one sample must be collected into Trizol and one into viral transport media (VTM). If only one sample can be collected, then place the sample into VTM.

Tissue specimens should be collected in triplicate. One specimen should be frozen in 500 µL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

Post-Mortem Blood Collection

From recently dead animals, it may be possible to collect whole blood (often clotted) from the right side of the heart where the largest volume of blood is available. Collect all available blood into an appropriate size container (typically one or more blood tubes). Allow the tubes to sit undisturbed for at least 30 minutes, and then centrifuge at high speed (2000 x G for 20 minutes). Transfer the serum (clear, yellow or red-tinged fluid at the top), preferably via pipetting, to appropriately labeled cryovials. Transfer the remaining blood clots to separate cryovials. Refrigerate or freeze both the serum and blood clots.

If a centrifuge is not available, allow the clots and cells to settle as much as possible, and then collect the serum and clots as described above. If the animal's death is recent enough that the blood has not yet clotted and a centrifuge is not available, invert the blood tubes after the blood has been collected to allow the clot to form on the rubber stopper. After the blood has clotted, turn the tube right side up and carefully remove the stopper with the adhered clot, thereby leaving a clean serum sample in the tube.

At a minimum, as many of the following blood samples as possible should be collected:

- 2 samples of 500 µL (**whole blood**) placed in 2 vials, one containing 500 µL **Trizol** and one containing 500 µL **VTM** (= maximum final ratio of 1:1). Mix each vial well.
- 2 or more aliquots (0.5 ml) of **separated serum**, frozen

Tissue Collection

Collect three, adjacent, approximately 200mg (pea-sized) samples of the following tissues:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Testes
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas
- Other, if required*

*It will usually require experience to identify abnormal tissues, but potentially recognizable gross lesions include masses, discolored areas, ulcerations, etc. Samples for histopathology (i.e., in formalin) should be collected at the abnormal margins to include both normal and abnormal sections in the same piece of tissue. Collection of any obvious internal parasites in ethanol is also recommended.

Section 5.2.11.e. References

Higgins, A. J., & Kock, R. A. (1984). A guide to the clinical examination, chemical restraint and medication of the camel. *The British Veterinary Journal*, *140*(5), 485–504.

Fowler, M. E. (2010). Chapter 4 Clinical Diagnosis: Examination and Procedures in *Medicine and Surgery of Camelids*. (3 edition). Ames, Iowa: Wiley-Blackwell.

http://www.dardni.gov.uk/safe_cattle_handling_guidance.pdf

http://www.fass.org/docs/agguide3rd/chapter05.pdf =

http://www.dpi.nsw.gov.au/agriculture/livestock/animal-welfare/general/livestock/sop/cattle/blood-collection

http://www.biotracking.com/goats/biopryn/use

Section 5.2.11.f. Appendix I. Dentition Age Determination for Cattle, Sheep, and Goats

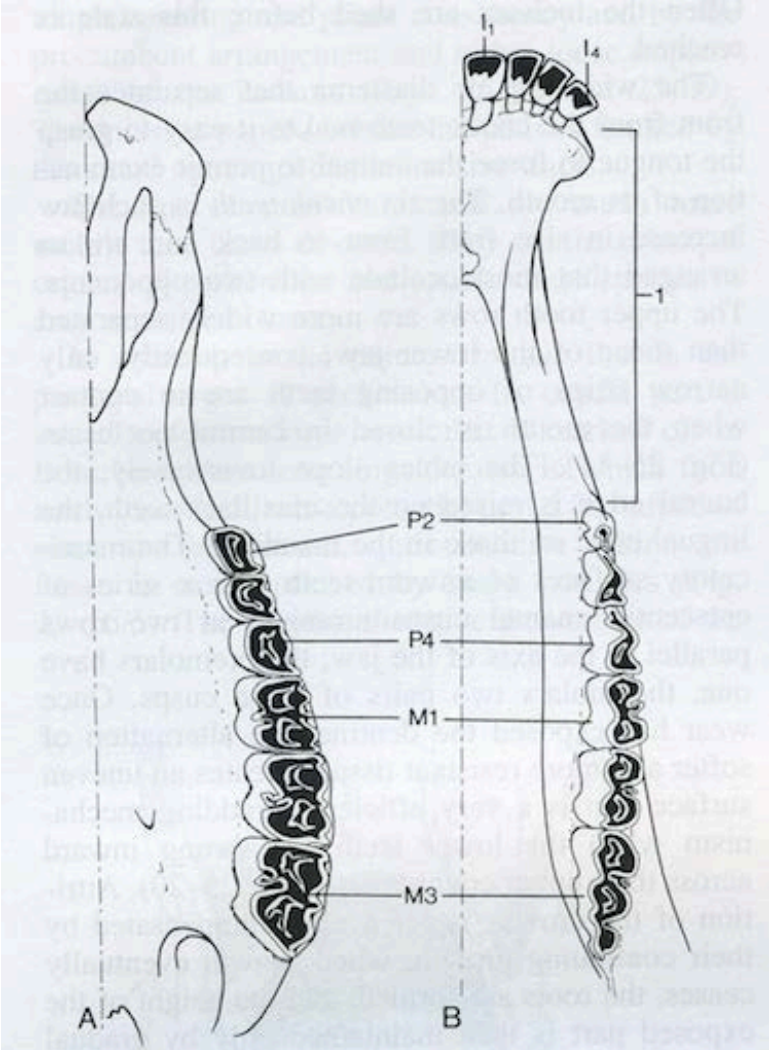

FIGURE 25–20. Left half of upper and right half of lower jaw of cattle. Notice the different shapes of the upper and lower cheek teeth and the large diastema (1).

Figure 1: From Dyce, Keith M., Wolfgang O. Sack, and Cornelis Johannes Gerardus Wensing. Textbook of Veterinary Anatomy. Elsevier Health Sciences, 2009.

Table 1: Eruption dates of the teeth of cattle

Teeth	Deciduous Teeth	Permanent Teeth
Incisor 1	Birth to 2 weeks of age	18 – 24 months
Incisor 2	Birth to 2 weeks of age	24 – 30 months
Incisor 3	Birth to 2 weeks of age	36 months
Incisor 4	Birth to 2 weeks of age	42 – 48 months
Premolar 2	Birth to 1 week	24 – 30 months
Premolar 3	Birth to 1 week	18 – 30 months
Premolar 4	Birth to 1 week	30 – 36 months
Molar 1		12 – 18 months
Molar 2		24 – 30 months
Molar 3		18 – 24 months

Table 2: Eruption dates of the teeth of sheep and goats.

Teeth	Deciduous Teeth	Permanent Teeth
Incisor 1	Birth to 1 weeks of age (at birth)	12 – 18 months
Incisor 2	Birth to 1 weeks of age (at birth)	18 – 24 months
Incisor 3	Birth to 1 weeks of age (at birth)	30 – 36 months
Incisor 4	1 to 3 weeks	36 – 48 months
Premolar 2	3 weeks	18 – 24 months
Premolar 3	3 weeks	18 – 24 months
Premolar 4	3 weeks	18 – 24 months
Molar 1	3 – 4 months	
Molar 2	8 – 10 months	
Molar 3	18 – 24 months	

Section 5.2.11.g. Appendix II. Snares

Figure 1: A commercial snout snare (left) and use of a modified snout snare, made from local materials, to restrain a pig during sampling in Bangladesh (right).

Section 5.2.11.h. Appendix III. Checklist for Supplies

General equipment and supplies

- ☐ Animal handling equipment – Halters and animal restraining ropes
- ☐ Data Collection forms
- ☐ Rubber stamp ink and pad
- ☐ GPS
- ☐ Camera
- ☐ Field Notebook
- ☐ Pen/Pencil
- ☐ Permanent markers
- ☐ Cryomarkers
- ☐ Protective clothing – Waterproof rubber boots, overalls, facemask, and nitrile gloves
- ☐ First aid kit
- ☐ Ice box containing ice packs (for short term storage and transport)
- ☐ Sharps bin
- ☐ Sturdy garbage bags
- ☐ Field centrifuge (portable 12vt)
- ☐ Liquid nitrogen dewar

Blood sample collection equipment and supplies

- ☐ EDTA vacutainer tubes – 9ml (lavender top)
- ☐ Serum separator vacutainer tubes – 9ml (red/gray top)
- ☐ Vacutainer needle holders
- ☐ Vacutainer needle: Cattle and Camels, 1½" 18 or 19 gauge; Sheep, Goats, and Swine, 1" 20G
- ☐ Syringes: 20, 10 and 5 ml
- ☐ Needles: Cattle and Camels, 1½" 18 or 19 gauge; Sheep, Goats, and Swine, 1" 20 guage for jugular or 22 or 23 gauge for auricular vein
- ☐ Alcohol (squirt bottle or vaporizer)
- ☐ Gauze
- ☐ Vacutainer tube rack
- ☐ Cryovial tubes
- ☐ Cryovial rack
- ☐ Centrifuge
- ☐ VTM
- ☐ Trizol

Fecal Sample Collection Equipment and Supplies

- ☐ Obstetrical Sleeve
- ☐ Disposable Spoons
- ☐ Plastic bags or vials
- ☐ Cryovial Rack
- ☐ Cryovials with VTM and Trizol

Urine Sample Collection Equipment and Supplies

- ☐ Plastic vials
- ☐ Plastic pipettes
- ☐ Cryovial Rack
- ☐ Cryovials with VTM and Trizol

Swab Collection Equipment and Supplies

- ☐ Plastic handle, polyester tip swabs
- ☐ Cryovial Rack
- ☐ Cryovials with VTM and Trizol

Tissue Collection Equipment and Supplies (in case of animal necropsy)

- ☐ 21 Gauge needles for cardiocentesis
- ☐ 1 mL Syringe for cardiocentesis
- ☐ Scalpel and surgical blades
- ☐ Forceps
- ☐ Sharp and blunt tip scissors
- ☐ Cryovial Rack
- ☐ Cryovials with VTM and Trizol
- ☐ Small Vials or Jars
- ☐ 10% Buffered Formalin

Section 5.2.11.i. Appendix IV. Additional Permit Requirements for Livestock Samples Imported into the United States

In addition to all other permits, livestock samples require special import permits from the USDA.

http://www.aphis.usda.gov/publications/plant_health/2012/fs_imp_food_ppq.pdf

http://www.aphis.usda.gov/wps/portal/aphis/resources/permits/!ut/p/a1/jZDLDoIwFES_hi0dKmJ1VyVCfUVjjNiNQYOVBKgpKL8vGjfG5-zuzTnJZIgkEZFFfElVXKW6iLPbLb2tKxilLVDBlgsPYjiftQeUOgi8Btg0wCDgoduZAHAZhfD7od_pTgHh_efjQzh--Wsin5HA4X7jLSezRTgExs4D-FbxDnzpMCJSZXp332PDi12LKSJNckhMYuyzad7HqjqVPQsW6rq2ldYqS-y9zu3YWHhnHXVZkegFJqd8FSGd52tW8ivrt9DV/?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_animal_health%2Fsa_import_into_us%2Fct_animal_imports_home