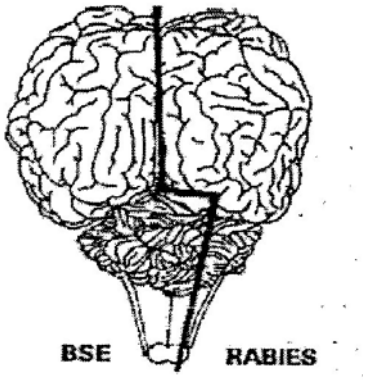
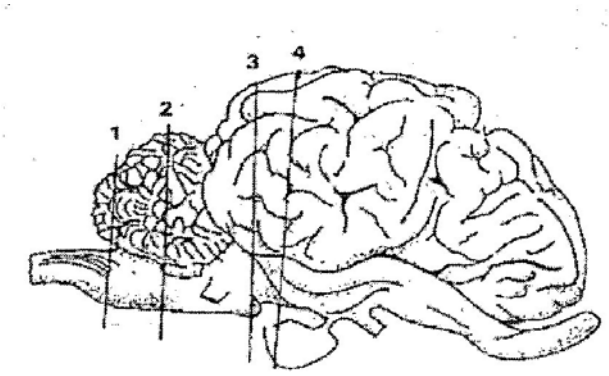
6.1 Appendix 1—Brain Sampling Techniques

BSE suspects and BSE surveillance cases that are concurrent rabies suspects:

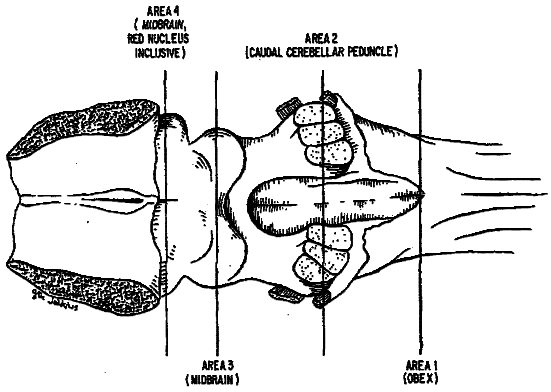
Collect and submit whole fresh brain bisected as detailed below. The lateral view diagram details four areas critical for the diagnosis of BSE, 1 and 2 being the most important. **Do not freeze or fix the samples in formalin**.





BSE surveillance cases if rabies is not a consideration:

Collect and submit fresh brainstem including the obex (indicated in Figure 1). **Do not freeze or fix the samples in formalin**.



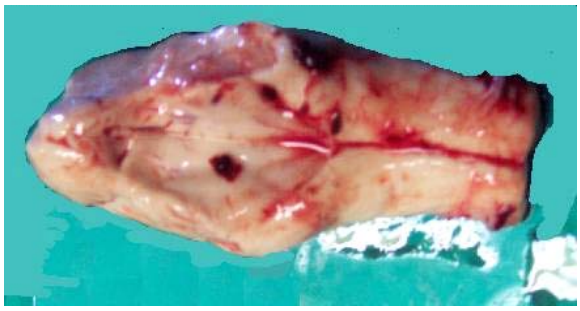


Figure 1

Brain Collection Protocols

The whole brain removal technique, the spatula technique, and the flushing technique are all suitable for BSE specimen collection.

Whole Brain Removal Technique

1. In instances where rabies cannot be ruled out, tissue samples should be submitted for both rabies and BSE testing. Place a disposable plastic drop sheet under the head of the carcass. The entire brain must be removed. This is to be done using a saw, knife, and chisel. DO NOT USE AN AXE TO OPEN THE CRANIUM. Ensure that the brainstem is removed intact.

2. Separate the head from the carcass at the atlanto-occipital joint and cut the spinal cord.

3. Place the disarticulated head on a table covered with a disposable plastic drop sheet.

4. Remove the skin from the skull and posterior nose, including the skin around the eyes.

5. Using a post-mortem saw, make a cut to extend from a point just posterior to the orbit of the eye to a similar point on the opposite orbit (refer to Figure 2). This cut should be about 2 cm deep and slanted in a backward direction.

6. Make two other cuts on each side of the cranium from the middle of the foramen magnum parallel to the foramen's lateral borders to a point 2 cm medial to the orbital rim. This cut should be angled about 45 degrees inward (refer to Figure 3).

7. Insert a heavy knife or chisel into the first cut and pry the top of the skull caudally. Care should be taken to prevent the attached meninges from disrupting the brain substance, especially the meninges between the cerebral hemispheres and anterior to the cerebellum. Scissors are more suitable than a knife to cut these membranes.

8. Cut the olfactory tract and raise the brain slightly upward so the optic nerve can be cut. As the brain is raised, the pituitary stalk comes in to view. Cut the stalk leaving the pituitary gland in its bony fossa.

9. Gently raise the brain upward and backward. Cut the cranial nerve roots. This allows the brain, along with a four centimetre segment of cervical spinal cord, to be freed from the cranial cavity.

10. Bisect the brain as depicted in Figure 1 above and submit the specimen for BSE testing and, if appropriate, for rabies testing.

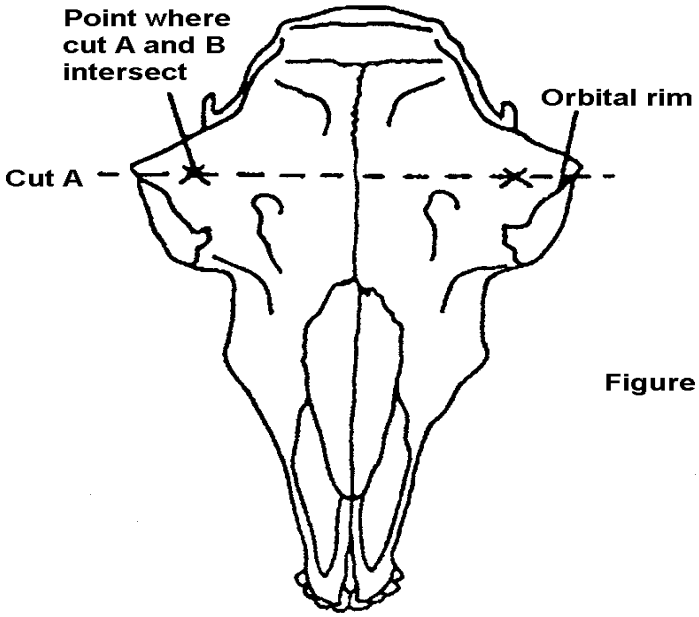


Figure 2

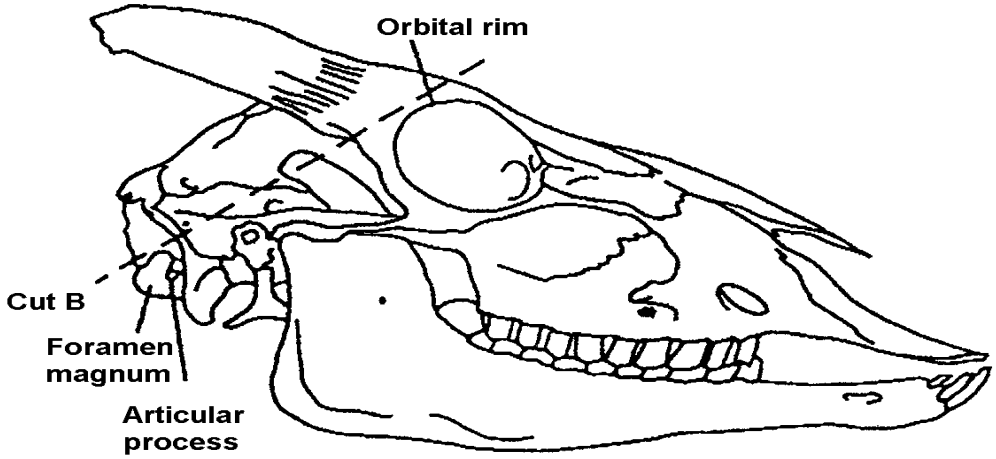


Figure 3

Spatula Technique

11. Using a post-mortem knife, disarticulate the head from the carcass at the atlanto-occipital joint.

12. Place the disarticulated head on a table or suitable work surface covered with a disposable plastic drop sheet. The head should be placed dorsal side down with the foramen magnum facing you. Using forceps and scissors, remove the dura matter through the foramen magnum opening to expose the medulla.

13. Insert a brain knife rostrally into the cranial cavity through the foramen magnum. Move the knife in a side-to-side slicing motion until the tip of the knife contacts bone. Ensure that the knife blade is parallel to the dorsal surface of the brainstem and ventral to the cerebellum. Rotate the knife blade and carefully push aside the cerebellum. Remove the knife.

14. Insert the spatula through the foramen magnum. Ensure that the spatula blade is facing laterally. Push the blade rostrally until the shaft of the spatula is approximately two thirds into the cranial cavity. This will correspond to the level to the pons-mesencephalic junction.

15. Rotate the blade ventrally and dorsally severing the brainstem.

16. Grasp the caudal end of the brainstem with rat-toothed forceps. Gently pull the spatula and the forceps caudally to cause the removal of the brainstem. Place the brainstem on a paper towel in the primary receptacle for submission to testing.

Spoon Technique

17. To perform this technique, a pair of mayo dissecting scissors, rat-toothed forceps, and an obex removal spoon are required.

18. Place the disarticulated head on a table or other suitable work surface covered with a disposable plastic work sheet. The head should be placed ventral side down with the foramen magnum facing you. Using the forceps and scissors, make a single cut down the centre of the exposed dura matter thereby creating two flaps.

19. Hold the dura matter with the forceps to expose the cranial nerves exiting the spinal cord. Sever the cranial nerves from the spinal cord. This is the most important step in freeing the spinal cord and facilitating the spoon technique removal of the obex.

20. The spoon should be inserted face down through the foramen magnum. It should be moved forward to lodge the tip of it between the cerebellum and the brainstem/spinal cord. Apply downward pressure on the spoon while moving it from side-to-side over the spinal cord to sever it from the rest of the brain.

21. Use the spoon to gently pull the severed brainstem/spinal cord through the foramen magnum. Place the brainstem on a paper towel in the primary receptacle for submission to testing.

Submission of BSE Suspect (Confirmatory Negative) Samples

For each submission, complete *CFIA 1528—Pathology Specimen Submission Domestic Use Only* in the Laboratory Sample Tracking System (LSTS). Under the "Reasons for Submitting" section of the form, check the box indicating disease control. Indicate if the animal is a clinical suspect or an epidemiological trace out / trace in and provide the age and a description of the clinical signs noted on ante-mortem examination of the animal. Include a description of any post-mortem findings.

Samples derived from animals categorized as BSE confirmatory negative cases must be packed and shipped in accordance with the packaging and documentation requirements for *Diagnostic Specimens UN3373* as set out by the Biohazard Containment and Safety Division.

Rabies samples must be packed and shipped in accordance with the procedures for *Infectious Substances UN2814*. If a specimen for BSE testing is being sent simultaneously with a specimen for rabies testing, both samples must be sent in accordance with the packaging and documentation requirements for *UN2814* specimens.

All BSE confirmatory negative samples are to be shipped to:

National Centre for Foreign Animal Disease
Canadian Food Inspection Agency
1015 Arlington Street
Winnipeg, MB R3E 3M4
Telephone: 204-89-2001 Facsimile: 204-789-2038.

Submission of BSE Surveillance Samples

Samples derived from animals categorized as BSE surveillance cases can be packaged and shipped in accordance with the packaging and documentation requirements for *Shipping Biological Samples—Non Regulated*.

Samples may be derived from animals that are rabies suspects as well as BSE surveillance cases. To facilitate both rabies and BSE diagnostic evaluations as expeditiously as possible, divide the brain as depicted on page *6.1–1*. Both portions of the brain are to be shipped fresh. Rabies samples must be packed and shipped in accordance with the procedures for *Infectious Substances UN2814* to the CFIA's Rabies laboratory (Alberta or Ottawa).

BSE surveillance specimens should be sent to the nearest laboratory on the list below. Clearly indicate on *CFIA 1528* that samples from the same animal/brain have been submitted for rabies evaluation. This will alert laboratory staff to the potential level of biohazard and ensure the appropriate handling of specimens.

- Ottawa Laboratory Fallowfield
  Canadian Food Inspection Agency
  3851 Fallowfield Road
  Nepean, ON K2H 8P9

- Lethbridge Laboratory
  Canadian Food Inspection Agency
  Township Road 9-1
  PO Box 640
  Lethbridge, AB T1J 3Z4

- St-Hyacinthe Laboratory
  Canadian Food Inspection Agency
  3400 Casavant Blvd. W.
  St-Hyacinthe, QC J2S 8E3

- National Centre for Foreign Animal Disease
  Canadian Food Inspection Agency
  1015 Arlington Street
  Winnipeg, MB R3E 3 M4

6.2 Appendix 2—Sanitary Precautions /Disinfectants

BSE is considered to be a human pathogen. Safe work practices are outlined in the *Common Procedures Manual, 1.6 Personal Safety Practices*. With large scale operations, one individual should be assigned the role of a safety officer to assure that workers adhere to safe work practices.

Wear protective clothing, gloves and face protection when collecting brain specimens. Always avoid direct contact with brain tissues. Personnel at tissue harvesting sites should take precautions to avoid ingestion of the agent.

A disposable plastic drop sheet on which to place the animal's head is recommended. The sheet should be large enough to cover the work area.

Chemical decontamination of equipment and work surfaces with sodium hypochlorite (NaOCl) at a concentration of 2% available chlorine, or sodium hydroxide (NaOH) at a concentration of 2 Molars, is recommended. Surfaces and equipment should be left wet (or soaking) with NaOCl or NaOH for at least one hour.

It is recommended that neurosurgical tools be soaked in NaOH for one hour, removed from the solution, and wiped with the NaOCl solution for ten seconds. Dry the tools as the NaOCl is corrosive.

- NaOH can be purchased from Fisher Scientific in crystal form. To make a 2 Molar concentration of NaOH, dilute 80 grams NaOH crystals in one litre of water and stir well.

- NaOCl can be prepared from industrial grade or commercially available bleach (such as Javex). Dilute the bleach to provide a final concentration of 2% (20,000 ppm) available chlorine. For example, most commercially available bleaches have 6% available chlorine listed on the label. In this case, mix one part bleach and two parts water (ratio 1:2) to attain the 2% concentration of available chlorine. Used disposable protective clothing, gloves and animal remains should be buried or incinerated.

Note: Other traditional disinfectants such as Virkon are **not** effective against prion agents. Disinfection of instruments must be done with either sodium hypochlorite or sodium hydroxide.

6.3 Appendix 3—Reporting Line Responder's Questionnaire

BSE SURVEILLANCE 1-866-400-4244

RESPONDER'S QUESTIONNAIRE

SECTION 1:

To be completed by Receptionist/Area Office first responder—responder explains that the call will be referred to the appropriate CFIA district office and that a veterinarian will call them back promptly.

Date: **Time:**

Name of Caller:

Caller's Telephone number:

District/Regional Office where call was referred:

Name of Contact at District/Regional Office:

Section 1 completed by: (name of responder)

SECTION 2:

To be completed by District Veterinarian and/or First Responder

ANIMAL HISTORY:

□ AGE

□ IDENTIFICATION NUMBER

□ SICK □ DOWN □ DYING □ DEAD

□ DATE & TIME OF DEATH

□ SYMPTOMS EXHIBITED, DISEASE COURSE, TREATMENT, HUMAN EXPOSURE

□ PROVINCIAL OR PRIVATE VET CALLED

□ CFIA DISTRICT OFFICE CALLED

PRELIMINARY VETERINARY ASSESSMENT:

□ RABIES OR BSE SUSPECT

□ MEETS BSE SURVEILLANCE CRITERIA

□ DOES NOT MEET BSE SURVEILLANCE CRITERIA

ACTION REQUIRED:

□ CFIA DISTRICT OFFICE TO ATTEND PREMISES

□ OWNER ADVISED ANIMAL INELIGIBLE FOR SURVEILLANCE SAMPLING

SECTION 3:

This section is for reporting purposes: Operations to determine nature of information required....

FOLLOW UP to be completed by District Office

Sample taken: Yes No

Date:

Positive Negative

Reimbursement information:

OFFICE INFORMATION

Completed by:

Date:

6.4 Appendix 4—Humane Euthanasia of Cattle

Euthanasia requires the animal to be rendered unconscious without distress or suffering before the cessation of vital life functions. Although several euthanasia techniques exist, they fall into one of the following categories:

- Physical disruption of brain activity caused by direct destruction of brain tissue (e.g. gunshot, penetrating captive bolt stunning followed by ex-sanguination, or other suitable techniques that cause death).
- Drugs that directly depress the central nervous system (e.g. anaesthetics, barbiturates) and induce death by hypoxia.
- Agents that induce unconsciousness followed by mechanisms that induce hypoxia (e.g. narcotics followed by ex-sanguination).

The method employed must provide for the following effects:

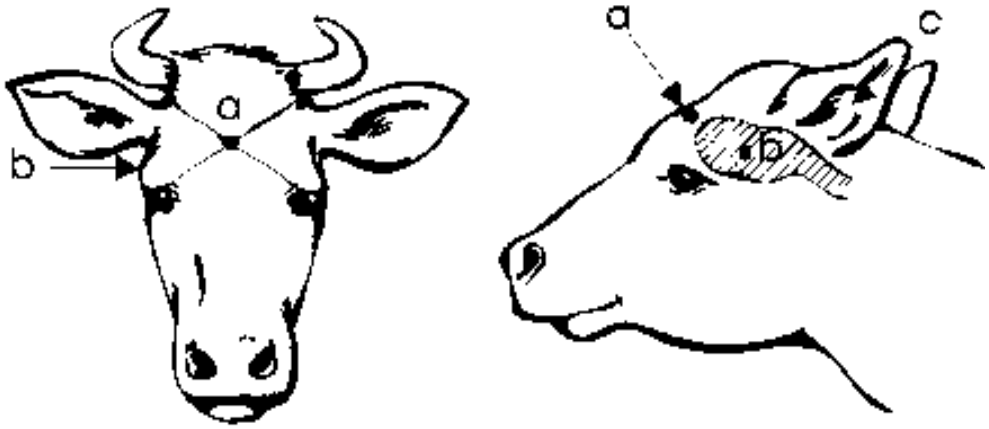
- stunning and rapid death, irreversible;
- death without panic or distress;
- reliable for both single animals and large numbers;
- minimal detrimental impact on operators and observers;
- disease control considerations (e.g. spillage of body fluids).

The skill of the operator is vital for the humane killing of animals.

Method	***Human Safety***	***Animal Welfare***	***Skill***	***Required Cost***	***Aesthetics***	***Considerations***
Gunshot Stunning	Moderate; firearm laws apply	Good	Moderate; correct placement essential	Low; after initial purchase	Moderate; some blood and body movement	Distance from animal can be maintained
Penetrating Captive Bolt Gun Stunning	Moderate	Good	Moderate; correct placement essential	Low; after initial purchase	Moderate; some blood and body movement	Contact with animal required
Barbiturate Overdose	Good	Excellent	Moderate; intravenous injections required	High	Good	Drug only available to licensed veterinarian
Ex-sanguination	Fair	Good; animal must already be unconscious	Moderate	Low	Poor; very bloody	Not sole method of euthanasia
Electrocution	Moderate to poor	Good; only if specialized equipment is used	Moderate	Low; initial purchase high	Fair; some body movement	Electricity required

Note: A non-penetrating captive bolt is unsuitable for cattle under field conditions.

Electrocution of adult cattle is difficult under field conditions and generally not recommended.



Locations for captive bolt/gunshot stunning of young and adult cattle

a) frontal method; intersection of two lines between the medial canthus of the eye and the base of the opposite horn (or just above the base of the ear).

b) temporal method (gunshot only); midway between eye and base of ear on same side; firing from the side, bullet directed horizontally.

c) from the top of the head: possible for young animals; not recommended.

Note: A captive bolt or gunshot alone do NOT reliably kill an animal.

Shotguns are very effective and, if used correctly, are safer than rifles and hand guns. Keep the muzzle 5–25 cm away from target area. Use #4, 5, or 6 birdshot. If the shotgun is of small calibre (such as a .410), use a powerful cartridge (such as a 3-inch magnum).

The shot strikes as a compact mass at high velocity and the pellets disperse after penetrating the skull. The entry wound is relatively small, but the brain damage is massive and results in the death of the animal.

There is little chance of shot exiting the carcass (safer than a free bullet).

Signs of an effective stun are as follows:

- animal collapses and becomes rigid;
- absence of rhythmic breathing;
- no corneal reflex; fixed “glazed” eye expression.

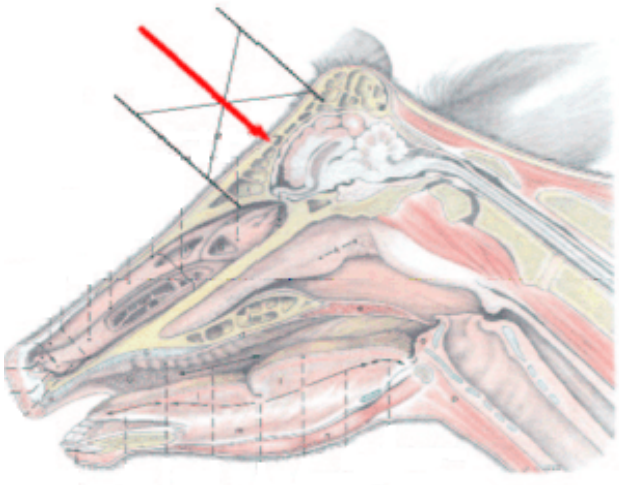
The foremost sign of an ineffective stun is the return of rhythmic breathing!

Note: Pithing is recommended as a standard procedure for (large scale) euthanasia of cattle, sheep, and pigs immediately following stunning. While proven to be effective and meeting animal welfare standards in the EU, the method has not yet been a topic in Canada. Pithed ruminant carcasses are considered unsuitable for human consumption.

The Animal Welfare Committee of CVMA is expected to take a position on pithing.

Note on BSE sample quality:

Neither the Western Blot nor the ELISA rapid test are influenced by the destruction caused by gunshot or captive bolt stunning. The penetration depth of

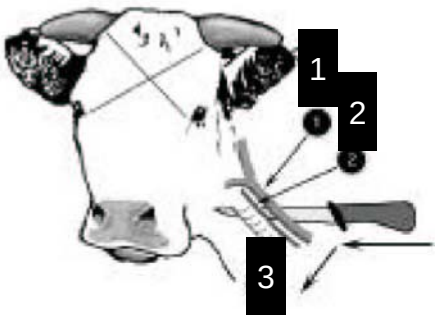


the captive bolt is approximately 8 cm. If placed and aimed correctly, this is not deep enough to cause destruction of the obex and brainstem that would be sufficient to render the sample useless.

A similarly aimed gunshot (frontal method) or a gunshot using the temporal method (bullet exits at the opposite temporal area) are both unlikely to cause an amount of destruction to the obex that would make the sample unusable.

Captive bolt and gunshot both cause an amount of haemorrhage that requires "cleanup" of the sample in the laboratory.

Shotgun pellets cause massive brain destruction and are likely to also destroy the brain stem tissue.



Ex-sanguination:

Use a sharp, pointed knife with at least a 15 cm rigid blade. Thrust the point of the knife into the neck just below the vertebral column and draw downward to sever the following:

1. external jugular vein
2. common carotid artery
3. trachea

Sources:

Practical Euthanasia of Cattle, Animal Welfare Committee of the American Association of Bovine Practitioners

Getting it right, DEFRA, 2003

Personal communication, CFIA Winnipeg Laboratory - Foreign Animal Diseases (Arlington)