



# National TSE Surveillance Project (NTSESP)

## National Guidelines for Field Operations

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2024-25

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## GLOSSARY

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ACDP	Australian Centre for Disease Preparedness, Commonwealth Scientific and Industrial Research Organisation, Geelong, Victoria
AHA	Animal Health Australia
AHO	Government Animal Health Officer (includes stock inspectors and government veterinarians)
ANZSDP	Australian and New Zealand Standard Diagnostic Procedure
AUSVETPLAN	Australian Veterinary Emergency Plan
BSE	bovine spongiform encephalopathy
CNS	central nervous system
DAFF	Australian Government Department of Agriculture, Fisheries and Forestry
NAHIS/NAHIP	National Animal Health Information System/ Program
NATA	National Association of Testing Authorities
NTSESP	National Transmissible Spongiform Encephalopathies Surveillance Project
PrP	prion protein
SAF	scrapie associated fibrils
TSE	transmissible spongiform encephalopathy
UK	United Kingdom
vCJD	variant Creutzfeldt-Jakob disease
WOAH	World Organisation for Animal Health (previously OIE)

## INTRODUCTION

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These Guidelines for Field Operations provide field and laboratory workers in both the private and public sectors with the information they need to participate in Australia's National Transmissible Spongiform Encephalopathies Surveillance Project (NTSESP). It contains background information on the need to undertake national surveillance for animal transmissible spongiform encephalopathies (TSEs) and describes the clinical signs and present understanding of the modes of transmission of BSE and scrapie.

The guidelines do not cover atypical scrapie, although cases of this rare disease in sheep may be an incidental finding from scrapie surveillance. The WOAH *Terrestrial Animal Health Code* Chapter 14.9 states that 'the chapter does not cover so-called 'atypical' scrapie, which is clinically, pathologically, biochemically and epidemiologically unrelated to 'classical' scrapie, may not be contagious and may, in fact, be a spontaneous degenerative condition of older sheep'.

The NTSESP provides for payments to be made to primary producers, private veterinarians and government agencies for submitting animals for examination where certain requirements are fulfilled. These requirements relate to case definition, specimen collection and documentation. The relevant requirements and their standards and criteria are described herein.

## TSE SURVEILLANCE IN AUSTRALIA

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### Background

Australia is recognised as meeting World Organisation for Animal Health (WOAH) requirements for a bovine spongiform encephalopathy (BSE) *Negligible Risk* and classical scrapie free country. Countries with this BSE and scrapie status must have implemented defined surveillance programs, as recommended by the WOAH, for both diseases. It is important that Australia meets this requirement to assure continued access to export markets.

Australia's status for BSE has been confirmed by national and international risk assessments - for example those conducted by the WOAH, Food Standards Australia New Zealand, the United States of America, and Japan.

Australia has operated passive and active surveillance programs for BSE and scrapie for many years. Passive surveillance occurs through routine investigations of disease by private and government veterinarians and stock inspectors, the ante - and post-mortem examination of all cattle, sheep and goats at slaughter, and the inspection of animals destined for live export.

Active surveillance for BSE was commenced in Australia in 1990 and was modified in 1997 under the NTSESP to comply with recommendations in the chapter on BSE and what was then the draft chapter on scrapie in the WOAH Terrestrial Animal Health Code.

The NTSESP is an integrated national project jointly funded by governments and livestock industries to demonstrate Australia's ongoing freedom from BSE and scrapie and to provide early detection of those diseases should they occur. The project is managed by Animal Health Australia.

The objectives of the NTSESP are:

- trade support - to maintain a TSE surveillance system consistent with the WOAH *Terrestrial Animal Health Code* that assures all countries that import cattle and sheep commodities from Australia can be assured that Australia remains free of these diseases
- protect public and animal health – to ensure the early detection of BSE should it occur in Australia's cattle so that an appropriate, early response can be mounted under AUSVETPLAN to protect the health of Australia's people and animals.

The NTSESP comprises:

- field investigations by government and private veterinarians of animals where there is suspicion of a TSE on the grounds of clinical signs
- veterinary pathologists screening the case histories of all laboratory submissions, with a clinical history of nervous disease, to consider whether they can exclude the diagnosis of a TSE
- veterinary histopathologists, trained in the diagnosis of a TSE, screening the brains of submitted specimens from cattle over 30 months and less than nine years of age, and from sheep and goats over 18 months of age (but preferably less than five years) with a clinical history of nervous disease to detect lesions of TSE
- further laboratory testing of screen test results that are not conclusively negative
- the collection of samples by Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) from fallen and casualty slaughter cattle, sheep and goats for testing with rapid TSE tests – see Appendix Three
- maintenance of NATA accreditation for rapid TSE tests by the Australian Centre for Disease Preparedness (ACDP, previously AAHL).
- participation in external proficiency testing for TSEs (ACDP)

- an easily accessible and up-to-date national database of TSE surveillance information supported by case records for at least 7 years
- publication of NTSESP results in the National Animal Health Information System's (NAHIS) quarterly newsletter - *Animal Health Surveillance*, its annual report - *Animal Health in Australia*, and on the Animal Health Australia website: [www.animalhealthaustralia.com.au/what-we-do/disease-surveillance/tse-freedom-assurance-program/surveillance-of-tses/](http://www.animalhealthaustralia.com.au/what-we-do/disease-surveillance/tse-freedom-assurance-program/surveillance-of-tses/).

Each state/territory animal health agency, and the commonwealth, participates in the NTSESP with a national coordinating role provided through the TSEFAP National Advisory Committee (NAC) and National Technical Committee (NTC). Awareness and training projects on TSE surveillance are carried out through industry peak bodies, state/territory animal health agencies and DAFF.

The NTSESP includes a financial incentive scheme for sheep and cattle to maximise reporting and investigation of eligible clinically consistent animals by producers and private veterinarians.

Eligibility criteria for the financial incentives are shown in Appendix 3 (fallen and casualty slaughter) and Appendix 2 (clinically consistent).

### National Surveillance Design

The NTSESP is structured to comply with the WOA *Terrestrial Animal Health Code* – for BSE, Chapter 11.5, and for scrapie, Chapter 14.9. The NTSESP involves submission of samples from *clinically consistent*<sup>1</sup>, *fallen*<sup>2</sup> and *casualty slaughter*<sup>3</sup> cattle and sheep.

Appendix 1 provides details about the application of the WOA TSE sampling principles to the NTSESP.

#### **BSE surveillance design (Appendix 1)**

The WOA has recently changed its surveillance guidelines for BSE. Previously *Negligible Risk* countries, like Australia, conducted *Type B surveillance* but now there is just the need for a targeted surveillance program. The design of Australia's BSE surveillance within the NTSESP, is shown in Appendix 1. Australia still samples from the three subpopulations - clinically consistent, fallen and casualty slaughter cattle.

As recommended by the WOA, the NTSESP is primarily focused on sampling *clinically consistent* cattle because this sub-population is recognised as the most sensitive sub-population to target and therefore **all clinically consistent cattle should be investigated**.

To ensure the funds of the NTSESP incentive scheme are used efficiently to promote submission of animals with the highest potential for TSE detection, incentive payments are only available for submission of *clinically consistent* animals that satisfy the eligibility criteria (Appendix 2).

The WOA considers seven years to be the 95<sup>th</sup> percentile of the BSE incubation period. Consequently, the likelihood of BSE is relatively low in cattle 8 years and older. Allowing for a margin of error in cattle ageing, **financial incentives are not available to cattle aged nine years or older**.

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<sup>1</sup> A *clinically consistent animal* is defined as "an animal that is found with clinical signs considered consistent with BSE". This is analogous with the term "clinical suspect" used in the WOA *Terrestrial Animal Health Code* Chapter 11.5 on BSE.

<sup>2</sup> *Fallen* cattle are defined by the WOA *Terrestrial Animal Health Code* Chapter 11.5 as "cattle over 30 months of age which are found dead or killed on farm, during transport or at an abattoir".

<sup>3</sup> *Casualty slaughter* cattle are defined by the WOA as "cattle over 30 months of age that are non-ambulatory, recumbent, unable to rise or to walk without assistance; cattle over 30 months of age sent for emergency slaughter or condemned at ante-mortem inspection".

### ***Scrapie surveillance design (Appendix 1)***

Scrapie surveillance meets the general requirements for countries that are historically free from scrapie, in accordance with the WOAHP *Terrestrial Animal Health Code* Chapter 14.9. It targets neurological cases and also includes sampling of fallen and casualty slaughter sheep (see below) and goats.

### **Field, laboratory and reporting procedures**

All procedures associated with the collection, processing, testing, reporting and invoicing of TSE submissions should be consistent with these guidelines.

Clinical and post-mortem examination of eligible animals is carried out by private veterinarians, and officers of state/territory animal health agencies and DAFF through existing networks and samples submitted to participating animal health laboratories. All TSE submissions must be accompanied by completed laboratory submission form/s.

Initial screen testing to specifically exclude classical TSEs is performed by veterinary pathologists and laboratory technicians trained in TSE diagnostic techniques. If required, further diagnostic investigations are undertaken by trained personnel located at ACDP.

The information obtained from eligible animals is recorded in NAHIS and presented as quantitative evidence to Australia's international trading partners of Australia's freedom from classical BSE and scrapie.

### ***Clinically consistent animal sampling (Appendix 2)***

A clinically consistent animal is defined as *"an animal that is found with clinical signs considered consistent with BSE"*. This is analogous with the term "clinical suspect" used in the previous WOAHP *Terrestrial Animal Health Code* on surveillance for BSE.

To claim financial payments from NTSESP incentive scheme, eligibility criteria for clinically consistent cattle and sheep must be met (Appendix 2), including that all submissions of clinically consistent animals must include a completed *'Clinical history and post-mortem report'* (Appendix 4).

Procedures for animal destruction, sample collection and dispatch, guidelines for the provision of producer and veterinary incentives, and details on laboratory diagnosis are included in Appendix 2

### ***Fallen and casualty slaughter sampling (Appendix 3)***

**Fallen stock** are defined by the WOAHP previous *Terrestrial Animal Health Code* as *"cattle over 30 months of age and less than nine years of age which are found dead or killed on farm, during transport, or at an abattoir"*.

**Casualty slaughter** cattle are defined by WOAHP as cattle over 30 months of age that are non-ambulatory, recumbent, unable to rise or to walk without assistance; cattle over 30 months of age sent for emergency slaughter or condemned at ante-mortem inspection.

Approximately 150 brainstem samples from fallen and casualty slaughter cattle (95), sheep (47) and goats (8) are collected at export abattoirs and submitted to ACDP.

To claim financial payments from NTSESP incentive scheme, eligibility criteria for fallen and casualty slaughter cattle, sheep and goats must be met (Appendix 3).

Procedures for animal destruction, sample collection and dispatch, guidelines for the provision of incentives, and details on laboratory diagnosis are included in Appendix 3

### **Laboratory examination**

Laboratory examinations must comply with the current version of the *Australian and New Zealand Standard Diagnostic Procedure – Transmissible Spongiform Encephalopathies*, available from <http://www.agriculture.gov.au/animal/health/laboratories/procedures/anzsdp>.

Brain specimens (paraffin embedded tissue blocks and fresh central nervous system (CNS)) for TSE surveillance must be retained until a report of TSE exclusion has been issued by the relevant laboratory, after which they may be discarded or destroyed in accordance with normal laboratory policy.

Where appropriate, examinations will be conducted to investigate differential diagnoses.

Animal health laboratory examination for TSEs includes:

- histopathological examination of selected brain sections, chosen because of the known distribution of BSE and scrapie lesions
- examination of extracts of brain by electron microscopy for TSE associated fibrils of the protease-resistant prion protein
- inoculation of suspect prion containing material into mice to detect development of TSE disease
- examination of histological sections using antiserum and appropriate histochemical markers for immunohistochemical demonstration of prion protein
- immunoblot using antiserum after detergent extraction and protease digestion removes the normal prion protein to identify abnormal prion protein
- enzyme linked immunosorbent assay (ELISA) analysis using antibodies to detect abnormal prion protein after protease digestion to remove normal prion protein

State/territory animal health laboratories must refer samples with non-negative TSE screen test results to ACDP for further examinations<sup>4</sup>.

Laboratory findings are reported to the submitter and to the state NTSESP coordinator or delegate.

Laboratories invoice Animal Health Australia through state NTSESP coordinators for the cost of laboratory examinations.

### **National Reporting**

The database is the responsibility of the NAHIP Database Administrator. The NAHIP Database Administrator has the responsibility of keeping it functional and providing summary data to NAHIP publications each quarter.

State NTSESP coordinators have responsibility to ensure that records are entered into the national database as results become available from animal health laboratories. State/territory primary records must be maintained for at least 7 years using existing systems.

The NTSESP summary database information can be found at:

[https://nahis.animalhealthaustralia.com.au/public.php?page=pub\\_home&program=1](https://nahis.animalhealthaustralia.com.au/public.php?page=pub_home&program=1)

### **Quality Assurance and Reporting Standards**

Veterinary laboratories performing screening and confirmatory TSE testing must hold relevant NATA accreditations.

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<sup>4</sup> A *suspect case* of BSE is defined by the AUSVETPLAN Manual for BSE as “An animal of the genus *Bos* (cattle) or *Bubalus* (buffalo) with history, clinical signs and histological changes consistent with BSE, until BSE is confirmed or excluded; or an animal with a positive result from a sensitive and specific screening test such as an ELISA for transmissible spongiform encephalopathies, until BSE is confirmed or excluded.”



Laboratory testing of *clinically consistent* cattle, sheep and goats is processed individually. Targets for providing final diagnostic reports for TSE submissions (from the final laboratory if serial testing) are: 50% of animals within 4 weeks from the date of sample collection, 90% within 8 weeks and 100% within 12 weeks.

Laboratory testing of *fallen* and *casualty slaughter* cattle, sheep and goats is batched. Typically, samples are processed in quarterly test runs using rapid TSE testing kits.

All (100%) diagnostic reports for *fallen* and *casualty slaughter* submissions should be finalised within 16 weeks of the date of collection.

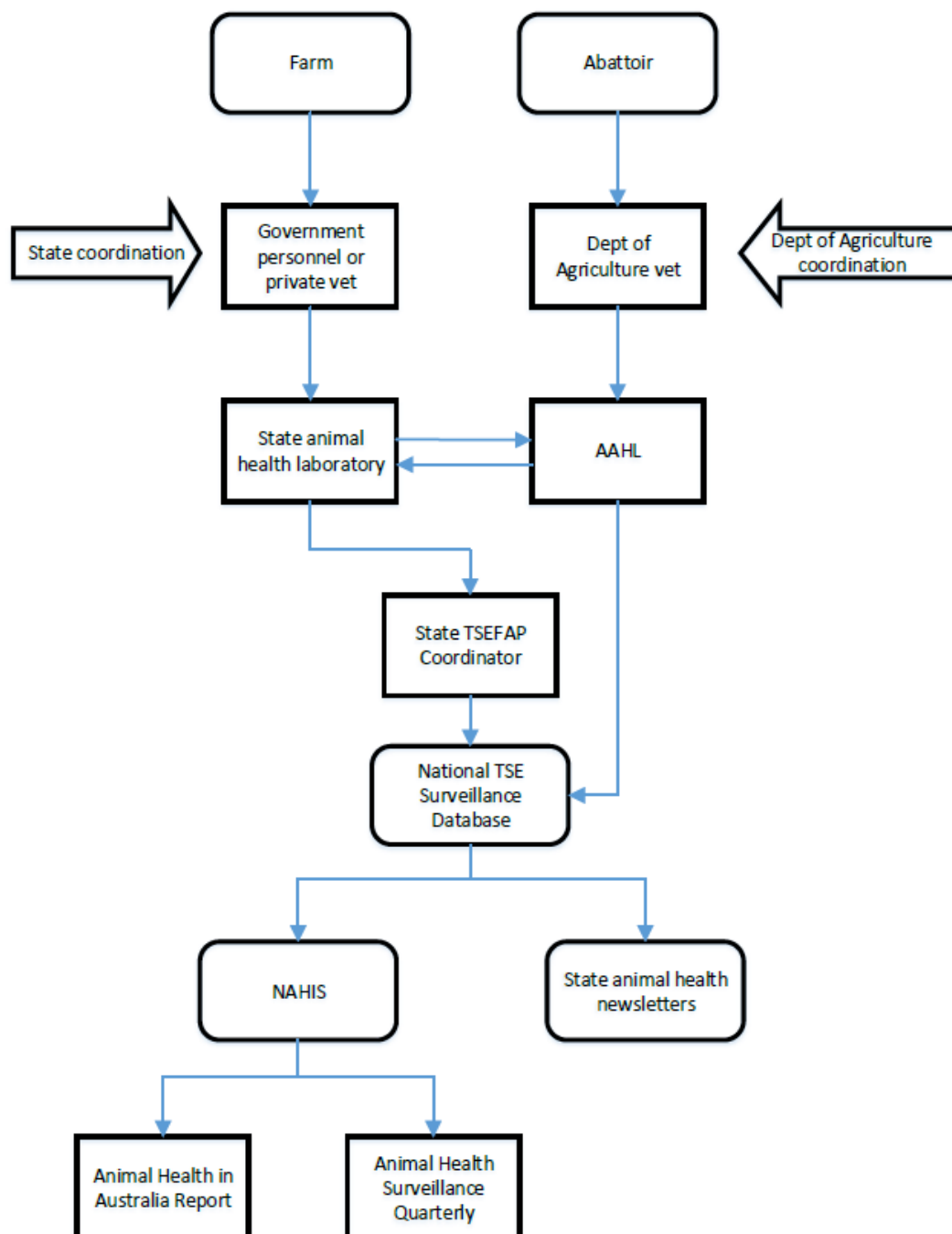


Figure 1: Specimen and information flows in the NTSESP

## BSE AND SCRAPIE: THE NATURE OF THE DISEASES

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BSE and scrapie are two of the TSE or “prion” diseases, which are characterised by progressive neurodegenerative disease of adult animals, long incubation periods and the accumulation in the CNS of an abnormal isoform of a host-encoded protein (PrP).

Three known strains of bovine spongiform encephalopathy (BSE) have been identified in cattle: classical BSE, low-type (L-type) BSE and high-type (H-type) BSE. L-type BSE and H-type BSE are also collectively called ‘atypical BSE’. Atypical BSE is a very rare disease that has been diagnosed in a number of countries.

### Aetiology

A protease-resistant isoform (PrP<sup>Sc</sup>) of a normal cellular prion protein (PrP<sup>C</sup>) has a pivotal role in the pathogenesis of TSEs and, according to the prion hypothesis, is the sole component of the TSE infectious agent. Scrapie has been recognised as a distinct neurological disease of sheep for centuries and is distributed widely in Europe and North America, with sporadic occurrence in some African and Asian countries.

BSE was first recognised in the UK in 1986, and the associated epidemic arose through the use of meat and bone meal (MBM) feed contaminated with the BSE agent. The origin of the BSE agent itself is uncertain, and various hypotheses have been considered. These include the possibility that BSE is derived from the scrapie agent, that it is a new prion agent from cattle or that it originated from some other mammalian species. Atypical BSE has been detected in a number of countries during large-scale surveillance for BSE in cattle. The origin of this rare condition is not yet known, but a spontaneous, non-contagious origin cannot be excluded.

### Clinical Signs

#### BSE

Due to the long incubation period, signs usually appear when cattle are older than 30 months of age, but less than nine years of age. BSE usually has an insidious onset and a slowly progressive clinical course extending over weeks to months. Apprehension, hyperaesthesia, and ataxia are the main signs, and at least one of these signs is present in most BSE cases; they represent the most frequent changes in mental status, sensation, and posture and movement respectively. Changes in mental status affect behaviour and temperament; the first sign of BSE may be when a normally placid animal becomes aggressive and kicks in the milking shed. Hypersensitivity can be to touch, sound and light. Ataxia affects mainly hind limbs. Other abnormalities of posture and movement include falling, tremor, and abnormal head carriage.

In advanced cases, generalised weakness and loss of condition can cause recumbency, and signs of altered mental status and hyperaesthesia may no longer be obvious but should be carefully sought in the clinical history of any recumbent animal. Loss of body weight and reduced milk yield often accompany the nervous signs as the disease progresses.

In BSE affected countries, BSE is considered in the differential diagnosis of “sudden” death or cases of purported misadventure. It is noteworthy that a higher incidence of BSE has been found in Europe in emergency slaughter cattle than in animals passing pre-slaughter inspection; when BSE has been diagnosed in either circumstance, there is often a history of overlooked clinical signs of BSE.

Most natural cases of atypical BSE have been reported in cattle that are at least 8 years of age. Clinical signs of atypical BSE (when present) can be similar to those of classical BSE; experimentally, they have included mental dullness and amyotrophy.

## Scrapie

A gradual onset of clinical signs is observed in sheep between two and five years of age. Signs observed may include hyper excitability; tremor, especially of the head and neck and in response to stimulation; pruritus leading to rubbing of wool and secondary skin trauma; and excessive nibbling or licking following tactile stimulation of the skin. In the later stages of the disease, ataxia, posterior paresis and constitutional signs such as emaciation or obesity may be noted. The clinical course can vary from weeks to months depending on the breed of sheep affected and the strain of scrapie agent.

Although scrapie in goats is considered rare, it has been reported in many countries. Most reports record contact with scrapie-affected sheep, but others indicate that scrapie can occur in goats without such contact. In one study (Sofianidis et al, 2006) clinical signs consisted of behavioural changes (e.g., hypersensitivity to normal external stimuli, hyperexcitability, bumping into objects, grinding of teeth, aggressiveness, listlessness, and a fixed facial expression) and motor changes (e.g., head tremor, hypermetrial movements, ataxia, weakness of the hind limbs, and tetraparesis) were observed. The animals tended to separate themselves from the rest of the herd when at pasture and some failed to return. Occasional signs included fainting episodes, abnormal bleating sounds, and low intensity pruritus in the region of the shoulder and lateral abdomen with continuous scratching against fixed objects or with the horns and hind limbs, resulting in localized alopecia and skin lesions. Restlessness, progressive emaciation and decreased appetite were described. The duration of the disease ranged from 2 to 6 months before the animals died or had to be killed.

## Pathology

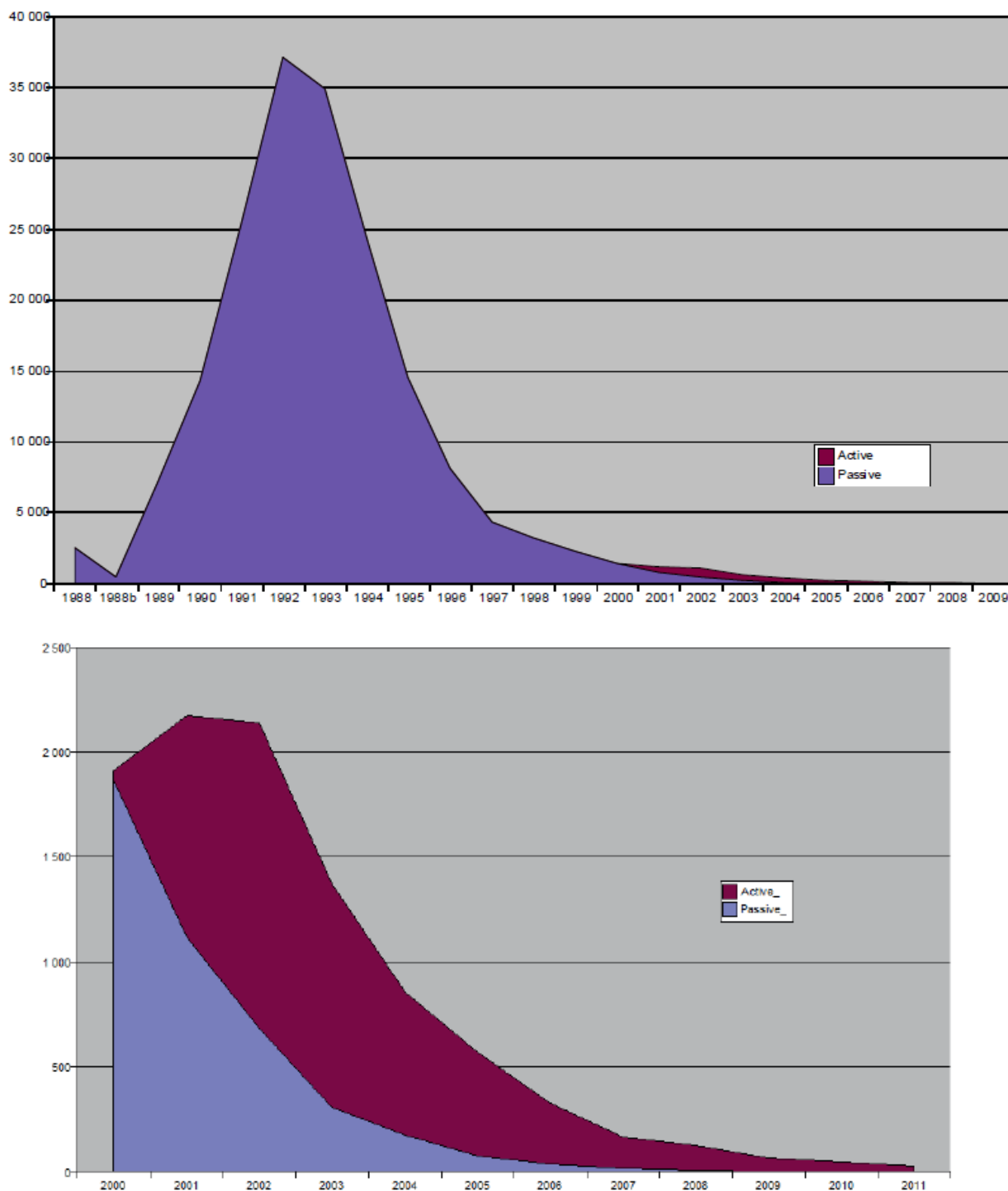
There are no gross changes in BSE or scrapie. The three characteristic histological TSE changes in the central nervous system (CNS) are vacuolation of grey matter neuropil (spongiform change) and /or vacuolation of neurons, astrocytosis, and neuronal degeneration. These changes have a predilection for certain neuroanatomical nuclei, particularly within the brainstem, and occur bilaterally and generally in a symmetrical distribution. These features of the pathological lesion mean that it is particularly important to submit the entire brainstem for histological assessment. Accumulation of PrP can be demonstrated within these lesions using immunohistochemical techniques.

## Modes of Transmission

### BSE

There is no evidence for maternal transmission or for horizontal spread of BSE between cattle, either directly or indirectly. This is consistent with the restriction of its infectivity largely to CNS tissue.

Epidemiological investigations suggest the BSE epidemic occurred as a result of ingestion of feed containing MBM contaminated with high concentrations of the BSE agent. Most cases were the result of calf-hood exposure to the agent. As carcasses of BSE-infected cattle were recycled through rendering plants, the contamination level in MBM and incidence of cattle infection were amplified, to produce the BSE epidemic. Since bans on the feeding of risk materials to ruminants were progressively introduced, BSE incidence in affected countries has dramatically declined (see figure 2) to the point where cases of classical BSE are rare now.



**Figure 2: BSE cases reported in the UK and Europe** (Source: Report on the monitoring and testing of ruminants for the presence of TSE in the EU in 2009 and 2011.)

The WOAHP recognises that BSE infectivity in cattle is largely confined to the brain, spinal cord, eyes, trigeminal and dorsal root ganglia, tonsils and distal ileum. The BSE agent is not known to be transferred in semen or embryos when collected to specified standards. TSE agents could potentially be spread by inoculation of biologically derived therapeutic products (iatrogenic spread) such as biological products derived from central nervous system extracts.

Fomites, including surgical and veterinary instruments have not been a recognised method of BSE spread to cattle in the UK outbreak. The potential for transmission of BSE by fomites is limited, because contamination requires exposure to CNS tissue from affected cattle. However, care is

required in the disposal or decontamination of equipment used for the post-mortem removal of brain tissue from suspected BSE cases. Surgical instruments used for procedures with CNS exposure (e.g. eye ablation) may also be contaminated if the animal is incubating BSE but such procedures are rare, and this form of transmission is very unlikely.

### **Scrapie**

The scrapie agent (PrP<sup>Sc</sup>) is found in medium to high titre in the brain, spinal cord, pituitary, spleen, tonsil, lymph nodes, ileum, colon, and rectum, of naturally infected sheep and goats. With scrapie, infection with the prion agent probably occurs via ingestion. In many cases this may be at or around the time of birth. The early appearance of the agent in tonsil, retropharyngeal and mesenteric lymph nodes and intestine suggests that oral infection plays a major role in primary infection; this could occur prenatally through ingestion of contaminated foetal fluids or postnatally via milk or a contaminated environment.

The pathogenesis of scrapie includes an initial replication phase in gut-associated lymphoid tissues before infection spreads to the central nervous system.

Susceptibility and resistance to the development of clinical scrapie is associated with several polymorphisms within the PrP gene.

Further information on BSE and scrapie can be found within their respective Australian Veterinary Emergency Plan (AUSVETPLAN) Disease Strategies<sup>5</sup>.

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<sup>5</sup> <https://animalhealthaustralia.com.au/ausvetplan/>

## APPENDIX 1 - SAMPLING DESIGN

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### Sampling Design for BSE

The NTSESP sampling design for BSE is based on Chapter 11.4 of the WOA *Terrestrial Animal Health Code*. Relevant sections from the chapter are modified and shown below.

Surveillance for BSE includes all bovines that show signs of the clinical spectrum of BSE.

In production and farming systems that allow bovines to be subjected to regular observation, it is likely that animals that display clinical signs suggestive of BSE will be more readily seen. Behavioural changes, which may be very subtle in the early clinical phase, are best identified by those who handle animals on a daily basis and who can monitor them closely for a progression of the signs. In production and farming systems, where bovines are not monitored as closely, situations may arise where an animal might be considered as a clinical suspect, yet if it has not been observed for a period of time, it may only be initially seen as unable to rise or walk without assistance or found dead (fallen stock).

The surveillance program should take into account that the vast majority of cases of BSE arise as single, isolated events. The concurrence of multiple animals with behavioural or neurological signs, or non-ambulatory or fallen stock is most likely associated with other causes.

The animals that lie on the clinical spectrum of BSE should be targeted for BSE surveillance and the following animals should be reported and followed up with appropriate laboratory testing in accordance with the *Terrestrial Manual* to accurately confirm or rule out the presence of BSE agents, including discrimination between atypical and classical BSE strains:

- a) those displaying progressive clinical signs suggestive of BSE that are refractory to treatment, and where the clinical presentation cannot be attributed to other common causes of behavioural or neurological signs (e.g. infectious, metabolic, traumatic, neoplastic or toxic causes);
- b) those showing behavioural or neurological signs at ante-mortem inspection at slaughterhouses/abattoirs;
- c) those unable to rise or walk without assistance, with an appropriate supporting clinical history (i.e. the clinical presentation cannot be attributed to other common causes of recumbency);
- d) those found dead (fallen stock), with an appropriate supporting clinical history (i.e. the clinical presentation cannot be attributed to other common causes of death).

### Sampling Design for Scrapie

The NTSESP is a formal program of targeted surveillance, which is consistent with the WOA *Terrestrial Animal Health Code*, 2023 recommendations for countries to be considered free from scrapie (Article 14.8.3, 2a).

Article 14.8.3, point 2a requires:

- i) scrapie has been notifiable for at least 25 years; and
- ii) a formal programme of targeted surveillance and monitoring, which includes testing of sheep and goats displaying clinical signs compatible with scrapie and those over 18 months of age slaughtered, culled or found dead on farm, can be documented as having been in place for at least 10 years; and
- iii) appropriate measures to prevent scrapie introduction can be documented as having been in place for at least 25 years; and
  - either scrapie has never been reported; or
  - no case of scrapie has been reported for at least 25 years.

The sampling design of the NTSESP is based on the requirements of a previous version of the WOAHP Terrestrial Animal Health Code chapter on scrapie and continues to meet the current chapter requirements.

The program was originally based on detecting scrapie with 99% confidence if it comprised 1% of neurological cases. It was assumed there were 80 million sheep in Australia and that 50 million of these would be over 18 months of age, resulting in a population of interest comprised of the 5,000 expected neurological cases from this group. This resulted in a recommendation to examine a minimum of 440 eligible neurological cases each year assuming perfect sensitivity and specificity of the diagnostic system.

The NTSESP continues to undertake targeted testing of a similar proportion of the current sheep population of Australia on an annual basis. While scrapie in goats would most likely be seen in Australia as a 'spill-over infection' from sheep, goats are now included in the NTSESP (albeit without incentives for cases).

## APPENDIX 2 TSE SURVEILLANCE IN CLINICALLY CONSISTENT CATTLE, SHEEP & GOATS

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### Incentive Eligibility Criteria - Clinically Consistent

Payments for submission (producer incentive, vet fee rebate, collect and document, and freight), TSE laboratory tests and other laboratory tests to investigate alternate diagnoses may be claimed from the NTSESP incentive scheme for *clinically consistent* cattle and sheep submissions that meet the eight incentive eligibility criteria below. *Clinically consistent* goat submissions are only eligible for the collect and document fee.

- **Species** – only cattle, sheep and goats are eligible for payment.
- **Live examination** – the animal should be examined while alive by the submitting veterinarian or biosecurity officer to independently establish the clinical state prior to euthanasia and sampling. Where this is not possible, video footage of the animal displaying clinical signs may be acceptable (if the veterinarian or officer has agreed to it).
- **Age** -
  - cattle must be at least 30 months but less than nine years of age
  - sheep and goats must be 18 months of age or older (preferably less than 5 years).
- **Clinically consistent animal** – the animal must have at least two clinical signs consistent with BSE or scrapie as per Appendix 4 (Clinical history and post-mortem report form).
- **Sample type and quality** – submitters must submit complete samples of the correct tissues (brain and spinal cord) of diagnostic quality for TSE evaluation to a participating laboratory as per these national guidelines.
- **Completion of required forms** - each submission must include completed documentation as per the policy and procedures of the relevant agency including a completed laboratory submission form and *Clinical history and post-mortem report* from (Appendix 4) or equivalent.
- **1 or 2 animals only** per disease incident per property.
- **Conflict of interest** – the submitting veterinarian or biosecurity officer must not have an actual or perceived conflict of interest with the recipient of a payment arising from the NTSESP (e.g. a financial or family link to them);  
or must satisfy the relevant state agency that a clinically consistent animal could not have been reasonably submitted by an alternative veterinarian or biosecurity officer without an actual or perceived conflict of interest.



## Differential Diagnoses of Nervous Disease in Australian Cattle and Sheep

It is important, wherever possible, to establish a diagnosis for the condition under investigation.

### Differential Diagnoses for Australian CATTLE Include:

#### - trauma

- brain and spinal cord
- musculoskeletal diseases
- nutritional myopathy (vitamin E or selenium deficiency)

#### - metabolic diseases

- hypomagnesaemia/ hypocalcaemia
- nervous acetoanaemia
- hepatic (e.g. pyrrolizidine alkaloidosis) and renal encephalopathy
- polioencephalomalacia
- heat stress

#### - infectious diseases

- brain or spinal abscess (including cranial or vertebral osteomyelitis)
- listeriosis
- thromboembolic meningo-encephalomyelitis
- cerebral babesiosis (type 1.3 – BHV 1.3)
- bovine herpes virus encephalitis
- sporadic bovine encephalomyelitis
- bovine malignant catarrhal fever
- focal symmetrical encephalomalacia (*Clostridium perfringens*)
- bovine ephemeral fever
- rabies (exotic)

#### - toxicoses

- lead toxicosis
- plant toxicoses
  - perennial rye grass staggers (*Acremonium* sp endophyte on *Lolium perenne*)
  - Annual rye grass staggers, blown grass staggers/flood plain staggers (*Clavibacter toxicus* on seed heads)
  - paspalum staggers (ergotism: *Claviceps paspali* on *Paspalum dilatatum*)
  - phalaris staggers
  - Swainsona* spp toxicosis
  - Zamia (palm) staggers
  - Xanthorrhoea* spp (grasstrees) toxicity
- botulism
- urea toxicoses

snakebite

**- genetic diseases**

cerebellar hypoplasia (Shorthorn, Brahman cattle)

cerebellar abiotrophy (Angus cattle)

progressive ataxia (Charolais cattle)

progressive spinal myelinopathy (Murray Grey cattle)

neuronal ceroid-lipofuscinosis (Devon cattle)

tomaculous-like neuropathy (Santa Gertrudis cattle)

**- neoplasia**

**Differential Diagnoses for Australian SHEEP<sup>6</sup> Include:****- pruritus**

lice, keds, itch mite, cutaneous myiasis

**- metabolic diseases**

hypomagnesaemia/hypocalcaemia

polioencephalomalacia

hepatic (e.g. pyrrolizidine alkaloidosis) and renal encephalopathy

copper deficiency

**- infectious diseases**

brain or spinal abscess

bacterial meningitis

listeriosis

meliodosis

focal symmetrical encephalomalacia (*Clostridium perfringens*)

**- genetic diseases**

ovine segmental axonopathy (Murrurundi disease, Mudgee ataxia)

cerebellar abiotrophy (Yass ataxia)

thalamic-cerebellar neuropathy

cervicothoracic vertebral subluxation and ataxia

**- toxicoses**

lead poisoning

plant poisoning

“humpy back” (suspected *Solanum esuriale*)

perennial rye grass staggers (*Acreonium* spp endophyte on *Lolium perenne*)

annual rye grass staggers, blown grass staggers/flood plain staggers (*Clavibacter* spp on seed heads)

paspalum staggers (ergotism: *Claviceps paspali* on *Paspalum dilatatum*)

phalaris staggers

Sorghum spp associated neuraxonal degeneration

*Tribulus terrestris* staggers (Coonabarabran ataxia), *Tribulus micrococcus* ataxia (Narrabri ataxia)

*Swainsona* spp toxicosis

botulism

Ixodes paralysis

delayed organophosphate toxicosis

urea poisoning

**- neoplasia**


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<sup>6</sup> Many of these differential diagnoses would also be applicable to goats

## INSTRUCTIONS FOR SUBMITTERS - CLINICALLY CONSISTENT

These instructions support assessment of cases, specimen collection and submission of *clinically consistent* animals to be examined, tested and reported under the NTSESP.

It is valuable to establish an alternate presumptive or definitive diagnosis in clinically consistent animals that do not have a TSE. As clinical signs consistent with TSEs may arise from lesions affecting tissues other than the brain, submitters should collect and submit a range of samples that will support a full laboratory investigation, and not simply exclude a TSE. It is recommended that submitters discuss what additional samples to submit with a veterinary pathologist.

**To receive incentive payments from the NTSESP, all submitters** (private veterinarians and government officers) **must ensure that each clinically consistent case meets the eligibility criteria.**

**Submitter assessment of cases as suitable for submission for TSE exclusion and eligible for incentive payments.**

While all cattle, sheep and goats that meet the WOAHP definition of 'clinically consistent' should be submitted for TSE exclusion – only those that meet all eight eligibility criteria are eligible for payments under the NTSEP financial incentive scheme.

Payments will not be made for TSE exclusion of ineligible animals unless otherwise agreed with AHA on a case-by-case basis.

Submitters must establish that the case is 'clinically consistent' with BSE or scrapie by carefully examining affected live animals and establishing a full history of the circumstances of occurrence of the disease, and any treatments and response to treatment.

Clinical assessment of 'nervous system disease' and 'clinically consistent' with BSE or scrapie should be considered in the broadest sense. Animals suitable for inclusion in the project may have subtle abnormalities like lip or ear paralysis, or more obvious general disturbances like head pressing or convulsions. Animals with signs that could be consistent with BSE or scrapie should be assessed as 'clinically consistent' – even though these clinical signs may be primary or secondary to disease involving other organ systems.

**CAUTION** - the likelihood of infection from *Salmonella*, *Listeria*, *Leptospira* or other zoonotic organisms requires all persons conducting post-mortem examinations to undertake appropriate precautions.

### Cattle - clinical signs consistent with BSE

Cattle may be submitted as 'clinically consistent' if at least two of the following clinically signs are verified by examination of live cattle by an appropriate veterinarian (private or government) or government officer.

<b>Mental status</b>	<b>Sensation</b>	<b>Posture and movement</b>
<ul style="list-style-type: none"> <li>• altered consciousness</li> <li>• apprehension</li> <li>• behaviour change</li> <li>• excitability</li> <li>• frenzy</li> <li>• hesitation at doors, gates, barriers</li> <li>• herd hierarchy change</li> <li>• moribund without evidence of infection or trauma</li> <li>• teeth grinding</li> </ul>	<ul style="list-style-type: none"> <li>• blindness</li> <li>• excessive licking of nose and flank</li> <li>• head rubbing or pressing</li> <li>• head shyness</li> <li>• hyperaesthesia (sound, touch)</li> <li>• hypoaesthesia (sound, touch)</li> <li>• kicking persistently when milked</li> </ul>	<ul style="list-style-type: none"> <li>• abnormal ear position</li> <li>• abnormal head carriage</li> <li>• ataxia</li> <li>• circling</li> <li>• falling</li> <li>• fetlock knuckling</li> <li>• paralysis/paresis</li> <li>• recumbency</li> <li>• tremor</li> </ul>

### Sheep and goats - clinical signs consistent with scrapie

Sheep may be submitted as 'clinically consistent' if at least two of the following clinically signs are verified by examination of live sheep by an appropriate veterinarian (private or government) or government officer.

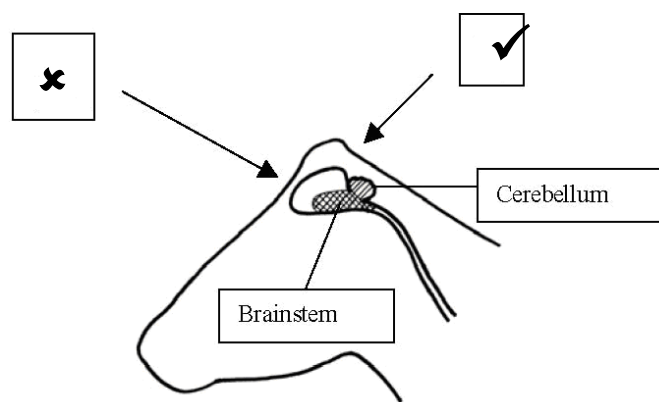
<b>Mental status</b>	<b>Sensation</b>	<b>Posture and movement</b>
<ul style="list-style-type: none"> <li>• altered consciousness</li> <li>• apprehension</li> <li>• behaviour change</li> <li>• frenzy</li> <li>• moribund without evidence of infection or trauma</li> <li>• temperament change</li> </ul>	<ul style="list-style-type: none"> <li>• blindness</li> <li>• hyperaesthesia (sound, touch)</li> <li>• hypoaesthesia (sound, touch)</li> <li>• rubbing/itching</li> <li>• wool or hair loss (flank and hind quarter)</li> </ul>	<ul style="list-style-type: none"> <li>• abnormal head carriage</li> <li>• ataxia</li> <li>• circling</li> <li>• falling</li> <li>• fetlock knuckling</li> <li>• paralysis/paresis</li> <li>• recumbency</li> <li>• tremor</li> </ul>

## Animal Destruction

The specimens are best removed from an animal killed by intravenous injection, commonly a barbiturate anaesthetic. Euthanasia performed in this way may constitute a danger to animals that might consume the carcass or offal, and this should always be borne in mind.

Alternative techniques such as captive bolt, shooting or the use of other injectable agents can be considered. A range of techniques are outlined in the AUSVETPLAN Destruction Manual. Care needs to be taken to avoid excessive damage to the cerebellum and brain stem. When shooting an animal, please consider the location of the brain in the skull (figure 3). A poll shot may be less likely to damage the brain stem than the routine frontal shot. Consult the relevant state/territory authority regarding recommendations for humane methods for animal destruction appropriate for the collection of diagnostic TSE exclusions specimens.

**In the case of a strong clinical suspicion of BSE or scrapie, it is essential the euthanasia method used enables collection of the whole undamaged brain and spinal cord samples.**



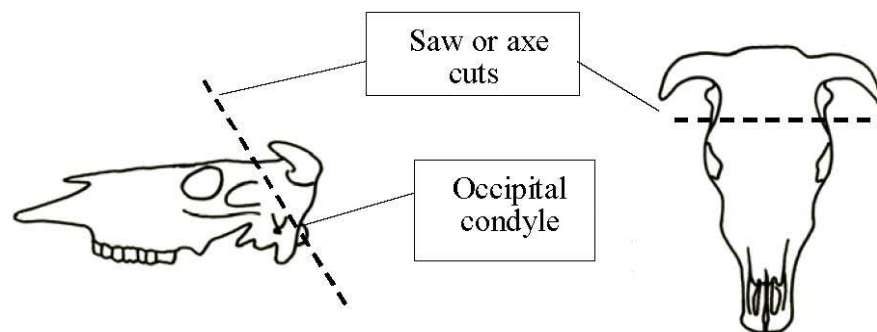
**Figure 3: Euthanasia procedure: consideration of cerebellum and brain stem**

## Brain Removal Techniques

There are a number of methods to remove the brain in the field without damaging the brain stem and cerebellum so that it is suitable for TSE examination.

**Method 1 - removing the skullcap.** This is achieved with 3 carefully placed cuts with a saw or axe (Figure 4). The two most important cuts are along the sides of the skull and must cut the occipital condyles. This allows the brainstem and first section of the spinal cord to be removed undamaged. The 3rd cut is placed transversely across the front of the skull, midway between the eye and the horn site. The lateral cuts must intersect with this transverse cut to form a triangle of cuts around the skull. The loosened skullcap can then be levered off using a sharpening steel or axe blade. After removing the dura mater (the white tough membrane that surrounds the brain) the brain can then easily be “rolled” out from front to rear using a boning knife to cut the nerve roots so that the caudal brainstem can be removed intact. Take particular care to remove all of the dura mater reflection (*tentorium cerebelli*) that projects downwards to provide a transverse partition between the cerebral hemispheres and cerebellum. This may ossify, particularly in cattle, and failure to excise it may result in damage to critical anatomic sites in the brainstem when the brain is subsequently lifted from the cranial cavity.

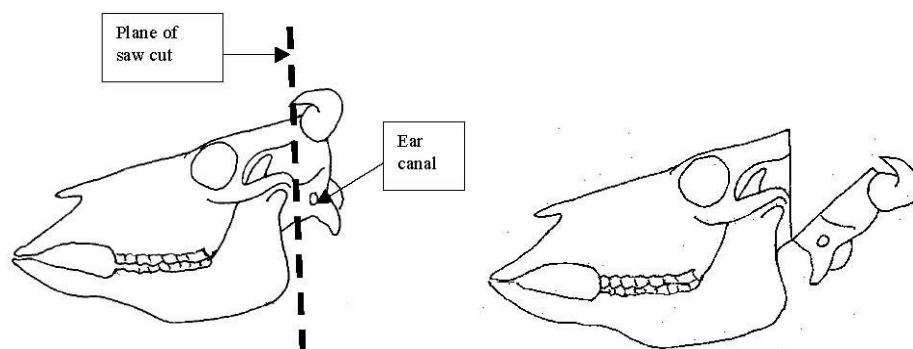
**Figure 4: Brain removal: cranial capping technique**



**Method 2 - removing the brain in pieces by cutting through the skull and brain transversely** with a saw (single transverse craniotomy). The cut is made dorsoventrally in a transverse plane that is perpendicular to the frontal surface, just cranial (1-1.5 cm) to the external ear canal, and parallel to the caudal border of the mandible as illustrated in Figure 5. The dorsoventral cut should angle forward rather than backwards.

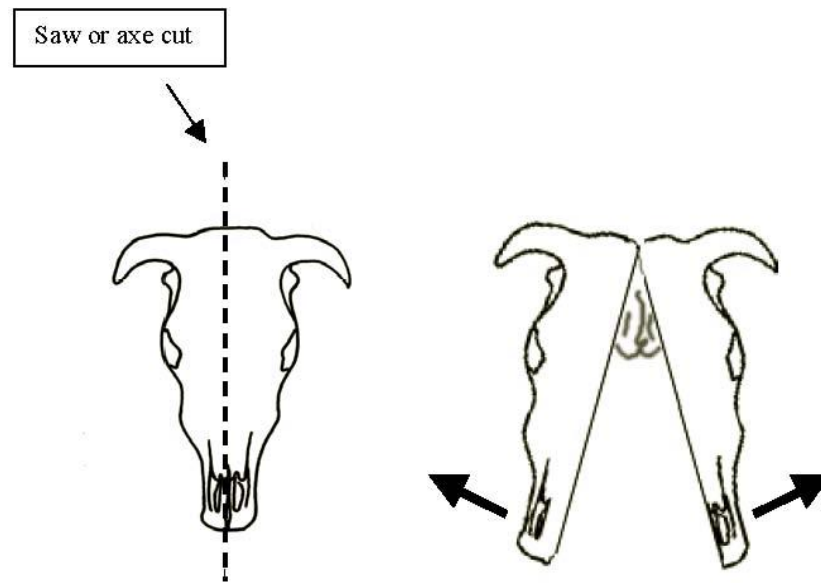
Note - laboratories discourage this technique because there is a risk that such a cut, if not placed sufficiently cranially, will damage a key diagnostic site in the brainstem. Also, if the cut is oblique, bilateral histological assessment is not possible at that site.

**Figure 5: Brain removal: transverse craniotomy technique**



**Method 3 - splitting the skull (but not the brain) ventrally and dorsally along its longitudinal axis** with a saw or axe (Figure 6). The axe can be hit with a small sledgehammer for greater control and safety. The two halves can be levered open from the nose end to expose the intact brain. This method requires practice. It has the advantage of exposing the pituitary gland. The brain **should not** be removed by longitudinally sectioning the skull **and** brain. Certain key diagnostic sites along the brainstem are located near the midline. These can be damaged with this technique as well as allow distortion of the brainstem during fixation and prevent bilateral histological assessment.

**Figure 6: Brain removal: longitudinal craniotomy technique**



These three techniques can be viewed at: <https://animalhealthaustralia.com.au/maintaining-australias-freedom-from-tses/> (under the TSE surveillance training section).

**Method 4 - submitting a whole head.** The head must be fresh, promptly chilled, and must **NOT** have been frozen. The weight and volume of the head can be reduced for chilling and transport by removing the mandible and front of the maxilla. To remove the front of the maxilla, make a transverse cut through the posterior edge of the bony orbits of the eyes.



## Specimens to Collect

### Essential specimens

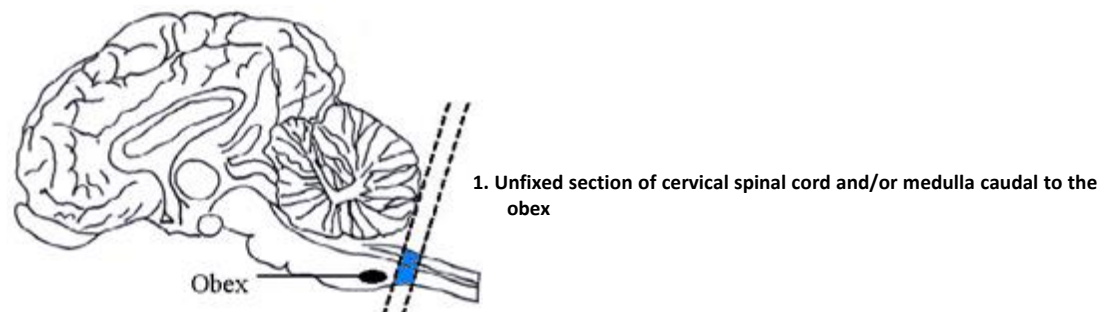
It is essential to submit both formalin fixed and unfixed (fresh) tissues from the central nervous system. Fixed brain tissue is used for histopathology and immunohistochemistry, which are the frontline tests to exclude TSEs. Unfixed cervical spinal cord (plus cerebellum for sheep) is collected in case testing of the fixed brain tissue cannot exclude TSE, and may be used for electron microscopy, immunoblot and mouse bioassay, as well as to further characterise a TSE if detected. In particular, the cerebellum of sheep is collected to facilitate further differentiation of classical scrapie and atypical/Nor98<sup>7</sup> scrapie by Western blotting if required.

The brain should be removed as soon as possible after death, with the brainstem attached and intact.

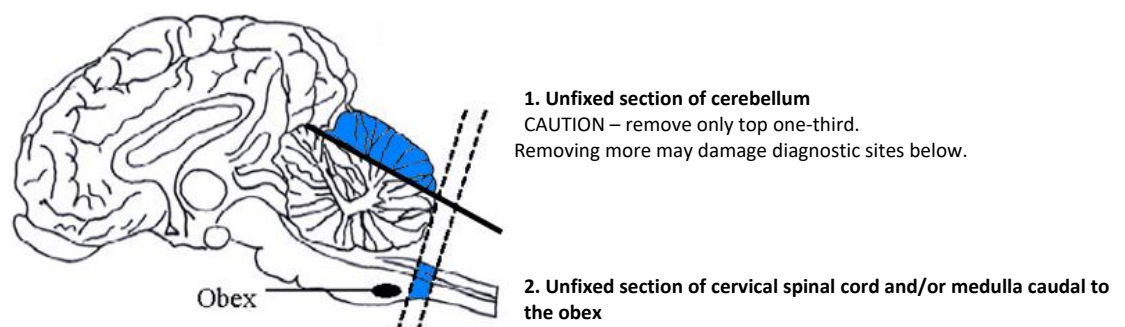
**Fresh tissues:** Samples of fresh brain should be collected and refrigerated without fixation, as soon as possible, either at refrigeration temperatures (4°C) or frozen (-20°C).

- **Cattle:** 2 - 3 cm length of cervical spinal cord and/or medulla caudal to the obex (see Figure 7a).
- **Sheep and goats:** (i) 2 - 3 cm length of cervical spinal cord and/or medulla caudal to the obex, **plus**  
(ii) the dorsal (top) third of the cerebellum sampled via a coronal/horizontal approach (CAUTION - remove only just the top third of the cerebellum, deeper sampling may damage TSE Standard Site 2 and compromise histological evaluation). See figure 7b.

These specimens must be collected and submitted for frozen storage at the state/territory laboratory at a consistent temperature of -20C or less. They should be retained until a report of TSE exclusion has been issued by the relevant laboratory, after which they may be discarded or destroyed in accordance with normal laboratory policy.



**Figure 7a: Cattle TSE exclusion – one unfixed tissue sample (blue shaded area)**

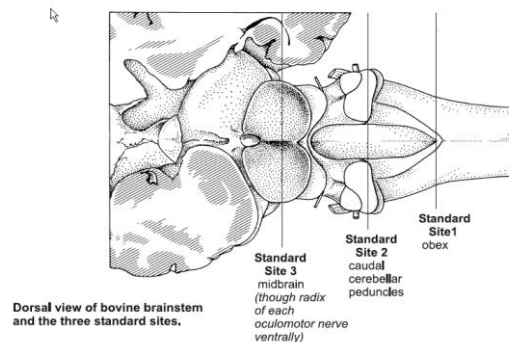


**Figure 7b: Sheep/goat TSE exclusion – two unfixed tissue samples (blue shaded areas)**

<sup>7</sup> Atypical/Nor98 scrapie was first identified in 1998 in Norway.

**Fixed tissues:** The remainder of the brain from cattle, sheep and goats, with residual cerebellum and brainstem attached, should then be submerged in 10% buffered formalin after removal. Prompt fixation helps to avoid artefactual vacuolation associated with decomposition that may mimic BSE/scrapie. It is important to ensure adequate volumes of 10% buffered formalin solution are used. Cattle brains need to be fixed in a minimum of 5 litres of 10% buffered formalin and sheep/goat brains in a minimum of 1 litre of 10% buffered formalin for a minimum of 7 days at room temperature (do not chill or freeze).

Use a sufficiently large container so that the brain “floats” and does not fix in a distorted position. The brain should be suspended with the cerebrum resting on the bottom of the container so that the caudal brainstem (midbrain and medulla) is not distorted through contact with the bottom of the container.



**Figure 8. Dorsal view of bovine brainstem and the three standard sites for histological exclusion of TSE (cerebellum has been removed in this view).**

### Optional specimens for laboratory investigations of differential diagnoses

Microbiological sampling of central nervous system tissue before fixation may be appropriate. Any other tissues with lesions should be preserved without delay in 10% buffered formalin.

The range of other specimens that ***should be considered*** (but are not mandatory) and the uses to which they may be put are as follows:

- formalin fixed tissues - slices of lumbar spinal cord, liver, kidney, heart and lung of a maximum thickness of 5 mm (can be fixed in the same container as the brain). Ensure adequate volumes of formalin are used.
- serum - serology (IBR, SBE, botulinum toxin), biochemistry (Ca, Mg, ketones),  
serum - bank (if required for future investigation)
- EDTA blood and smear - haematology, plasma cholinesterase
- organ smears of brain, kidney - tick fever organisms
- faeces - bacteriology, helminth egg and oocyst count
- fresh liver and kidney - lead, copper assay
- gastrointestinal content - rumen (botanical analysis), small and large intestine (botulism toxin) 2x50 ml samples

In addition to these standard samples, other samples may be appropriate on a case-by-case basis. If you are not sure which would be the most appropriate specimens for laboratory analysis, please telephone a pathologist at your veterinary laboratory.

## Documentation

Adequate documentation of the case details and history is required to meet national and international reporting obligations and would be critical in a response to detection of a TSE.

All required forms must be completed for the case to be eligible for payments from the NTSESP financial scheme.

The required forms include:

- laboratory submission form provided by the approved state/territory laboratory
- Clinical history-post mortem report (Appendix 4) or equivalent as provided by the relevant state/territory.

## Dispatch of Specimens

Ensure that:

- the specimens are securely and correctly packaged for transport
- all relevant details accompany the specimens, especially the required laboratory submission form and the *Clinical history and post mortem report* (Appendix 4)
- the laboratory is notified if the specimens have to be picked up from transport terminals after regular hours, as this may be essential to prevent deterioration of specimens.

**Fresh Specimens** - some fresh/chilled specimens (i.e. for microbiological culture, blood for biochemistry and haematology) should be dispatched on the day of collection to ensure handling at the laboratory as soon as practicable after death. The specimens must be sent with the laboratory submission form and the *Clinical history and post mortem report* (Appendix 4) with a clear indication that fixed specimens will follow.

The cervical spinal cord and/or medulla caudal to the obex, and for sheep/goats the dorsal third of the cerebellum, if kept chilled can be submitted with the fixed brain a few days after collection.

**Fixed Specimens** - to avoid transporting heavy and dangerous volumes of formalin, adequately fixed tissues can be kept moist during transport by placing fixed tissues in a securely tied, double plastic bag with either:

- 50 ml of 10% formalin, or
- wrapped in formalin-soaked high absorbency paper towels

The brain and other fixed specimens should be sent to the laboratory with a submission form but indicating on the submission form that fresh specimens had been previously sent.

## Guidelines for the Provision of Producer Incentives

### Background

Producers will be paid incentives for sheep and cattle by participating state/territory agencies under the NTSESP.

Incentives are provided to encourage the reporting and investigation of suitable animals and the submission of suitable specimens for examination. It is essential that producers recognise and report cattle and sheep with suspicious signs to a private veterinarian or government officer.

The producer incentive is available from the NTSESP via the relevant state/territory agencies to producer/s/owners for reporting and permitting euthanasia and sampling of cases that meet the *Eligibility Criteria for Clinically Consistent Cattle and Sheep* (see page 18). No payment will be made if the incentive eligibility criteria have not been met, except as agreed with AHA.

### Producer Incentives

\$330 per eligible cattle

\$110 per eligible sheep.

### Administration

A claim for payment of the TSE incentive should be made through a government animal health officer using an official claim form approved by the Chief Veterinary Officer of the relevant state or territory.

## Guidelines for the Provision of Veterinary Incentives

### Background

Private veterinarians and government agencies<sup>8</sup> may claim payment under the NTSESP for conducting post-mortem examinations and submitting reports and diagnostic samples. The payments assist government agencies to cover costs and provide incentive to veterinary practitioners to ensure suitable and appropriate reports are presented, specimens are collected, packaged and submitted to an approved laboratory to enable TSE exclusion and establish a likely alternative diagnosis so national targets for testing are met.

Veterinary incentives are available from the NTSESP for cases that meet the *Incentive Eligibility Criteria for Clinically Consistent Cattle and Sheep* (see page 16). No payment will be made if the incentive eligibility criteria have not been met, except as agreed with AHA.

### Veterinary fee rebates

\$340 (GST ex) for eligible cattle (\$240 for vet fees (cattle) and \$100 collect and document)

\$220 (GST ex) for eligible sheep (\$120 for vet fees (sheep) and \$100 collect and document)

A \$125 collect and document fee is payable for eligible goat samples.

Freight of \$30 (GST ex) for each eligible animal.

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<sup>8</sup> Submitters include private and government veterinarians and animal health officers.

## Administration

Private veterinarians must claim for payment of the TSE rebate through a government animal health officer using an invoice or the relevant claim form provided by the relevant agency.

State/territory agencies submit claims for veterinary incentives to AHA.

## Guidelines for the Provision of Veterinary Laboratory Incentives

### Background

Payments are available to government agencies under the NTSESP for laboratory investigation of eligible 'clinically consistent' sheep and cattle. The payments assist government agencies to cover costs and provide incentive to test for TSEs and investigate differential diagnoses in clinically consistent animals so national targets for testing are met.

Veterinary laboratory incentives are available from the NTSESP for sheep and cattle cases that meet the *Incentive Eligibility Criteria for Clinically Consistent Cattle and Sheep* (see page 18).

No payment will be made if the incentive eligibility criteria have not been met, except as agreed with AHA.

### Veterinary laboratory Incentives – State/Territory laboratories

Actual costs for TSE histopathology, for sheep, cattle and goats.

Actual costs for laboratory investigation of differential diagnoses, for sheep and cattle.

### Veterinary laboratory Incentives – ACDP

- TSE IHC: \$345 per test
- TSE ELISA: \$129 per test
- TSE Western blot: \$181 per test

### Administration

Claims for payment of laboratory costs should be submitted to AHA.

## Histological Diagnosis and Reporting of Clinically Consistent Animals

### TSE Positive

Characteristic vacuolation of grey matter neuropil (spongiform change) and/or of neurons, usually with a bilaterally symmetrical distribution. Other forms of neuronal degeneration and an astrocytic reaction support the diagnosis, if associated with grey matter vacuolation.

#### *Scrapie*

Neuronal vacuolation, particularly in the dorsal vagal nucleus at the obex, is usually more common than neuropil vacuolation. Occasional vacuolated neurons (1 or 2 in a section of medulla) without associated spongiform change in grey matter neuropil may be found in normal sheep brains.

#### *Bovine Spongiform Encephalopathy*

Neuropil vacuolation, particularly in the solitary tract nucleus and spinal tract nucleus of the trigeminal nerve at the obex, and in the periventricular grey matter of the midbrain, is more prominent than neuronal vacuolation, which is most frequent in the vestibular nuclear complex. In well-preserved material, a positive finding consists of more than 3 neuropil vacuoles at a neuroanatomical profile area.

Neuronal vacuolation in the red nucleus is a common incidental finding in normal cattle brains.

Diffuse vacuolation of white matter (myelinic vacuolation) is not a feature of natural scrapie or BSE. Distinction must be made between true spongiform change within grey matter, and vacuolation, which is an artefact of fixation or processing.

### TSE Pending

These are specimens with equivocal vacuolation of grey matter neuropil and/or neurons.

### TSE Unsuitable Specimen

These are specimens with severe autolytic change or inadequate representation of the standard sites for TSE exclusion, or of the neuroanatomical profile areas at these sites.

Where “TSE unsuitable specimen” is reported to the submitter, they should be notified that submitted tissues will be subjected to further testing and results advised as soon as possible but may take some weeks.

To minimise the number of TSE unsuitable specimens with the associated delays and extra laboratory costs, it is advisable that advice and extension materials on specimen collection be made freely available to submitters through their local government animal health staff.

### TSE Negative

These are specimens with no vacuolation of grey matter neuropil or neurons at the three standard sites.

### TSE Confirmatory Testing

Cases identified histologically as “TSE Positive”, “TSE Pending”, or “TSE unsuitable specimen” and lacking lesions in brain or other organs to account for the neurological signs, should be further tested at ACDP by immunohistochemistry as well as other diagnostic techniques if required.

### Report of TSE Exclusion

In all cases of progressive neurological disease in animals where classical TSE lesions have been excluded by histological examination of the standard brain sites, the laboratory report should include one of the following statements of TSE exclusion depending on histology screening results:

*“TSE Negative - No histological lesions suggestive of transmissible spongiform encephalopathy (TSE) detected at the brain sites specified in the Australian and New Zealand Standard Diagnostic Protocols for Animal Diseases –Transmissible Spongiform Encephalopathies.”*

OR

*"TSE negative - histological lesions with a low degree of suspicion for Transmissible Spongiform Encephalopathy (TSE) were detected at the brain sites specified in the Australian Standard Techniques for Animal Diseases - TSE's; specifically, the medulla at the level of the obex, the medulla through the caudal cerebellar peduncles and midbrain through the rostral colliculus. Samples were provided to ACDP for further testing, with classical TSE's excluded by immunohistochemistry on specified sites".*

OR

*"TSE negative - histological lesions with a moderate degree of suspicion for Transmissible Spongiform Encephalopathy (TSE) were detected at the brain sites specified in the Australian Standard Techniques for Animal Diseases - TSE's; specifically, the medulla at the level of the obex, the medulla through the caudal cerebellar peduncles and midbrain through the rostral colliculus. Samples were provided to ACDP for further testing, with classical TSE's excluded by immunohistochemistry on specified sites."*

## APPENDIX 3 - TSE SURVEILLANCE IN FALLEN AND CASUALTY SLAUGHTER CATTLE, SHEEP AND GOATS

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### Overview

Each year, brainstem samples from *fallen* and *casualty slaughter* cattle (95), sheep (47) and goats (8) are collected at Australian export abattoirs. These samples are submitted to ACDP and contribute to meeting WOAHP surveillance requirements.

### Incentive Eligibility Criteria - Fallen and Casualty Slaughter

Submission (collect and document) and TSE laboratory test costs may be claimed from the NTSESP incentive scheme for *fallen* and *casualty slaughter* submissions that meet all the incentive eligibility criteria below.

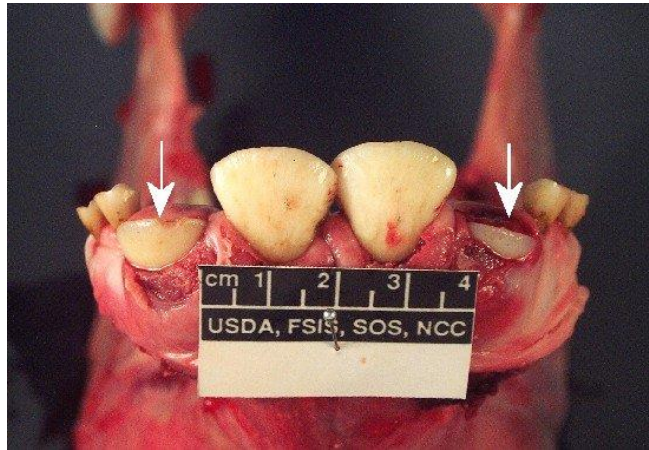
- **Species** – only cattle, sheep and goats are eligible for payment.
- **Age - Cattle**
  - at least 30 months but less than nine years of age, or
  - with middle (I1) permanent incisors erupted and in wear plus at least one of the second incisors (I2) erupted (see photograph below), but less than nine years of age.
- **Age – Sheep and goats**
  - at least 18 months of age or older (preferably less than 5 years); or
  - for sheep - with middle (I1) permanent incisors erupted and in wear plus at least one of the second incisors (I2) erupted.
- **Class of animal**, either
  - *fallen stock* as defined by the WOAHP sampled at an abattoir i.e. found dead or killed at an abattoir; or during transport to an abattoir
  - *casualty slaughter* as defined by the WOAHP sampled at an abattoir i.e. non-ambulatory, recumbent, unable to rise or to walk without assistance, sent for emergency slaughter or condemned at ante-mortem inspection.
- **Sample type and quality**
  - samples (as described in Appendix 1) of diagnostic quality are submitted to ACDP.
- **Adequate documentation**
  - owner and animal identification information has been provided for tracing purposes
  - all relevant laboratory submission form/s have been completed in full.

### NOTE

In the case of clinical suspicion of BSE or scrapie, or if the animal meets the eligibility criteria for a *clinically consistent* submission, every effort should be made to submit samples as a clinically consistent (rather than fallen or casualty) submission, as per Appendix 2, including collection of fresh and fixed undamaged brain and cord samples and other relevant samples for laboratory investigation of alternative diagnoses.



### Cattle of eligible age by dentition



**Figure 9:** An example of dentition of an animal meeting the eligibility criteria showing middle (I1) incisors fully developed and in wear and both I2 incisors erupted. Even if this animal had one of its I2 incisors erupted, it would meet minimum age eligibility criteria.

## BEEF AND VEAL LANGUAGE SUMMARY

### BASIC CATEGORIES

	<b>Veal ..... V</b>	<b>0 PERMANENT INCISORS</b> Female or entire male with no S.S.C. 0-70 kg H.S.C.W. 70.1-150 kg when carcass shows youthfulness and pinkish flesh
	<b>Beef ..... A</b>	<b>0 - 8</b> <b>0 - 8 PERMANENT INCISORS</b> Greater than 70 kg H.S.C.W. Female or Castrate male or Entire male with no S.S.C. of any age
	<b>Bull ..... B</b>	<b>0 - 8</b> <b>0 - 8 PERMANENT INCISORS</b> Entire male or castrate male bovine showing S.S.C.

S.S.C. = SECONDARY SEXUAL CHARACTERISTICS

H.S.C.W. = HOT STANDARD CARCASS WEIGHT

### ALTERNATIVE CATEGORIES

	<b>YEARLING BEEF ..... Y</b> <b>YEARLING STEER ..... YS</b>	<b>0</b> <b>0 PERMANENT INCISORS</b> * Up to 18 months
	<b>YOUNG BEEF ..... YG</b> <b>YOUNG STEER ..... YGS</b>	<b>0-2</b> <b>0-2 PERMANENT INCISORS</b> * Up to 30 months
	<b>YOUNG PRIME BEEF ..... YP</b> <b>YOUNG PRIME STEER ..... YPS</b>	<b>0-4</b> <b>0-4 PERMANENT INCISORS</b> * Up to 36 months
	<b>PRIME BEEF ..... PR</b> <b>PRIME STEER... PRS</b> <b>OX (female) ..... S</b>	<b>0-7</b> <b>0-7 PERMANENT INCISORS</b> * Up to 42 months
	<b>OX (male) ..... S</b> <b>STEER ..... SS</b> <b>COW ..... C</b>	<b>0-8</b> <b>0-8 PERMANENT INCISORS</b> Any age <b>8 PERMANENT INCISORS</b> Over 42 months

\* CHRONOLOGICAL AGE AS SHOWN IS APPROXIMATE ONLY

**Figure 10:** Beef and veal language summary

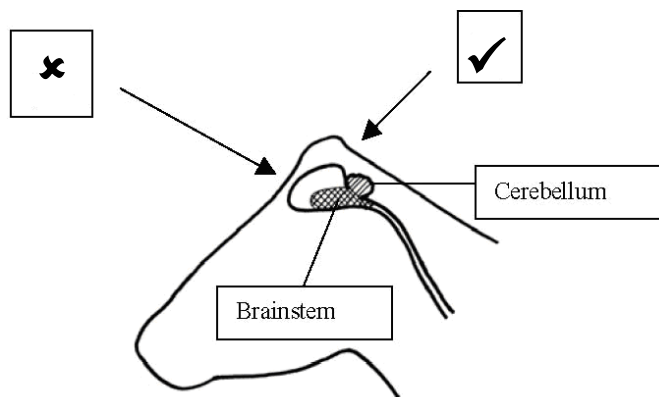
## INSTRUCTIONS FOR SUBMITTERS - FALLEN AND CASUALTY SLAUGHTER

### Animal Destruction

The specimen is best removed from an animal killed by intravenous injection, commonly a barbiturate anaesthetic. Euthanasia performed in this way may constitute a danger to animals that might consume the carcass or offal and the producer should be advised on the correct method of disposing of the animal e.g. by burial or complete burning.

Alternative techniques such as captive bolt, shooting or the use of other injectable agents can be considered. A range of techniques are outlined in the AUSVETPLAN Destruction Manual. Care needs to be taken to avoid excessive damage to the cerebellum and brain stem. When shooting an animal, please consider the location of the brain in the skull (Figure 11). A poll shot may be less likely to damage the brain stem than the routine frontal shot. Check with your state/territory authority regarding their recommendations for appropriate humane methods for animal destruction to collect brains for this project.

**In the case of a strong clinical suspicion of BSE or scrapie, it is essential to collect the whole undamaged brain and spinal cord samples (refer to Appendix Two).**



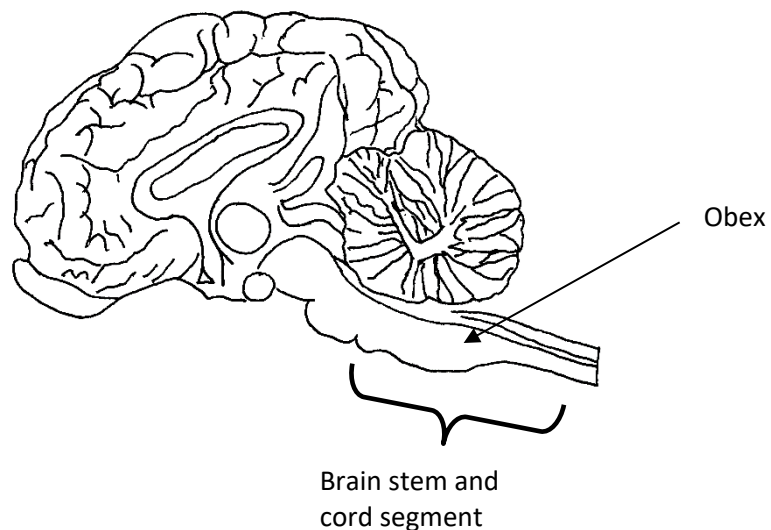
**Figure 11: Euthanasia procedure: consideration of cerebellum and brain stem**

### Sample collection and storage - Procedure for fallen and casually slaughter submissions

The preferred sample for ELISA testing, and subsequent confirmatory testing where indicated, comprises a portion of the brainstem including the obex. The sample is collected from the detached head using a modified spoon via the foramen magnum. Cleaning and decontamination of the spoon between samples is not essential. Where brainstem specimens are not available, such as might occur following certain methods of euthanasia, anterior cervical spinal cord is also acceptable.

Samples should be stored at refrigeration temperatures (4°C) prior to dispatch to the laboratory.

If sample receipt at the primary testing laboratory is expected to take longer than 3 days after the death of the animal, then the sample should be stored at freezer temperatures (-20°C or lower) prior to dispatch.



**Figure 12: Segment of brainstem and cranial spinal cord required for sampling fallen and casualty slaughter cattle and sheep.**

### Documentation

All samples must be accompanied by the relevant specimen submission form (e.g. ACDP Specimen Advice Note. See p38).

**IMPORTANT** - The form should be completed fully, and the owner and animal details confirmed before the samples are submitted. In the event of a positive result this information will be vital for international reporting and follow up investigations.

### Despatch of specimens

When reasonable and appropriate, samples should be dispatched to arrive at the primary testing laboratory within three days of the death of the animal.

Do not send samples to arrive at the laboratory out of business hours or at the weekend without checking with the laboratory first to ensure that they can be received. Liaise with the laboratory regarding the most appropriate time to send samples.

Specimens must be securely packaged and kept chilled during transport in accordance with national guidelines. The laboratory must be notified if the specimens have to be picked up from transport terminals after regular hours, as this may be essential to prevent deterioration of specimens.

### Diagnosis and reporting

One of four outcomes will be reported against submissions. The four outcomes are:

1. Unsuitable specimen: The specimen was unsuitable for examination under the fallen and casualty slaughtered stock project in the view of the duty veterinary pathologist. Reasons may include a tissue sample being too small, the way the specimen has been preserved (may have putrefied in transport), etc.
2. TSE Pending: This is an interim result indicating that more test results are to follow.
3. TSE Negative: The result of the rapid screening test is
  - a. Negative; **OR**
  - b. The screening test is positive or inconclusive, but the confirmatory testing is negative.
4. TSE Positive: The testing indicates that the sample contains material consistent with BSE infection with the screening test being positive and confirmatory testing also positive.

### TSE Confirmatory testing

Cases identified as “TSE Positive”, “TSE Pending”, or “TSE unsuitable specimen” should be further tested at ACDP by other diagnostic techniques.

### Report of TSE Exclusion

In all cases where a classical TSE has been excluded, the laboratory report should include one of the following statements of TSE exclusion as appropriate:

***“TSE Negative – the result of the rapid screening test is negative”.***

*OR*

***“TSE Negative – the result of the rapid screening test is positive or inconclusive, but the confirmatory testing is negative”.***




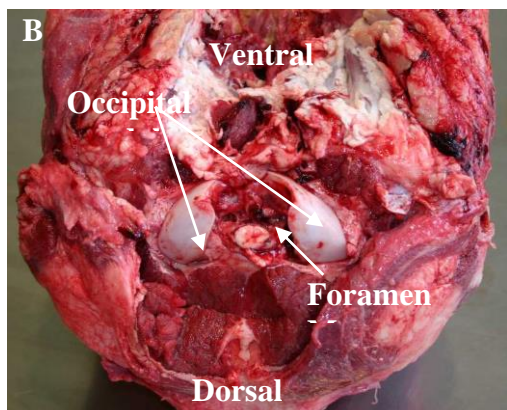
SPECIMEN ADVICE NOTE <i>Australian Animal Health Laboratory</i>		AAHL Use Only
Customer Barcode (if applicable)	CSIRO Animal, Food and Health Sciences (CAHFS) <b>Australian Animal Health Laboratory (AAHL)</b> Private Bag 24, Geelong, Vic 3220 Telephone: + 61 3 5227 5000 Fax: + 61 3 5227 5555 Email: <a href="mailto:aaht-accessions@csiro.au">aaht-accessions@csiro.au</a> Web: <a href="http://www.csiro.au/dsr">www.csiro.au/dsr</a>	  
<b>Submitter Reference:</b> <input type="text"/>	<b>Submission Category (circle one):</b> 1 = Routine Submission 2 = Emergency Disease Exclusion (low probability) 3 = Emergency Disease Exclusion (high probability)	
<b>Import Permit Number:</b> <input type="text"/>	<b>Owner:</b> Name: Town/Suburb: Postcode: State/Country:	
<b>Submitter (please include clinic/company name):</b>  	<b>Animal Details:</b> Species: Breed: Sex: Age:                      Years <input type="text"/> Months <input type="text"/>	
<b>State/Country:</b> 	<b>Clinical signs, PM findings, reason for testing:</b>	
<b>Email:</b> <input type="text"/>		
<b>Telephone (business):</b>		
<b>Telephone (after hours):</b>		
<b>FAX:</b>		
<b>Forward Account to:</b>		
<b>Send additional copies of reports to:</b>		
<b>Specimen Details (Number and type, including microchip and reference numbers as applicable):</b>  		
<b>Examination requested:</b>  		
<b>Date specimens collected:</b>	<b>Date of dispatch to AAHL:</b>	
<b>Date of export (optional):</b>		
<b>Signature of submitter (please print name underneath):</b>	<b>Date specimens received at AAHL:</b> (AAHL to complete)	

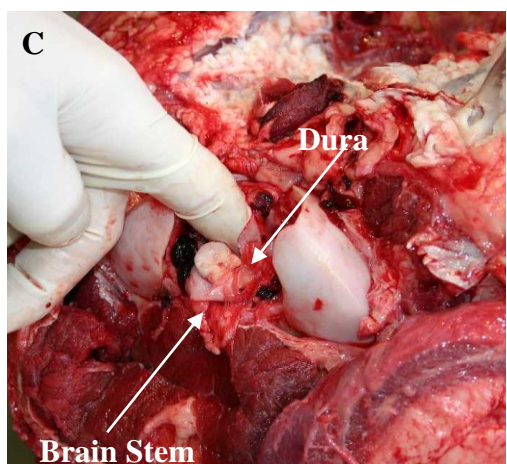
FIGURE 13: Sample Submission Form for ACDP



Prionic sampling involves the removal of the brainstem from the head using a specially modified spoon called a 'prionics spoon' as seen in Figure A.



Place the head on a flat stable surface with the dorsal surface facing down. Clear any excess muscle or fat away from the occipital condyles to allow easy access to the foramen magnum.



Separate the dura mater and any connective tissue from the brainstem before the prionics spoon is inserted. An index finger, as in Figure C, can be used.

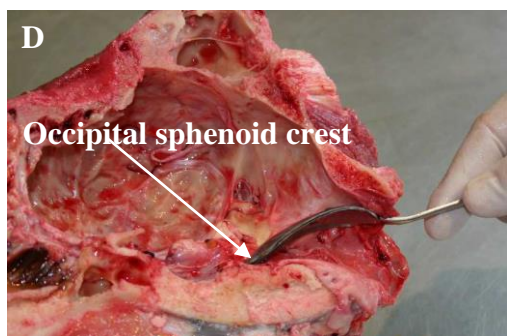
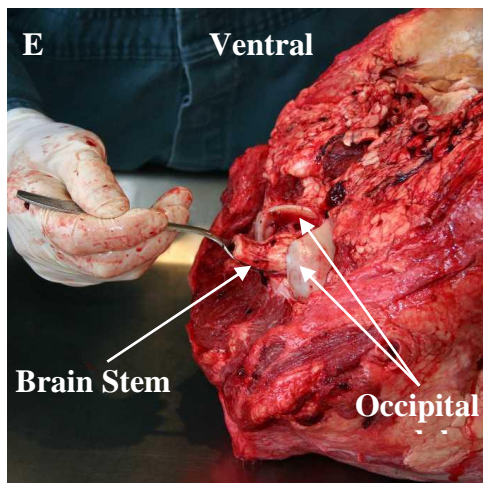


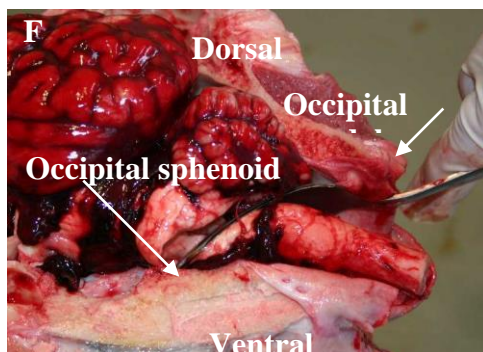
Figure D shows final placement of spoon. It should be placed firmly, caudal to the occipital-sphenoid crest. If placed on the rostral surface of the crest, severing and removal of the brainstem is very difficult.

**Figure 14: Sampling Fallen and Casualty Slaughter Cattle**





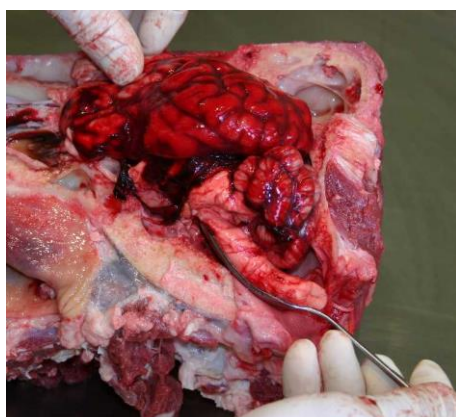
Insert the spoon slowly between the dura mater and the dorsal surface of the brain stem, until the junction of the handle and blade is level with the occipital condyles.



Once the blade is fully inserted, push the blade forward ventrally and firmly against the occipital-sphenoid crest and rotate from left to right to sever any connective tissue. To remove the brainstem, drag the spoon caudally along the ventral surface.



The brainstem should be made up of a small section of the spinal cord, obex and part of the medulla and should be placed in a yellow top container, details recorded on the side and chilled to 4°C, or frozen to less than – 20°C as soon as possible if receipt at the primary testing laboratory is expected to be longer than 3 days from the death of the animal.

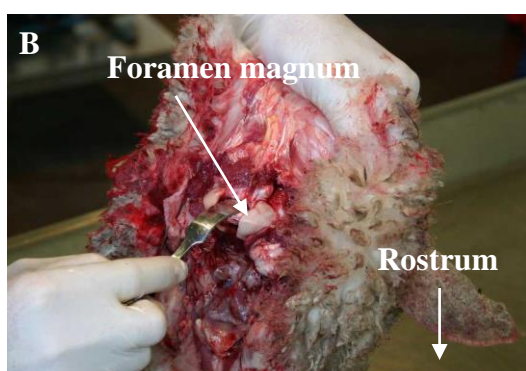


#### INCORRECT TECHNIQUE

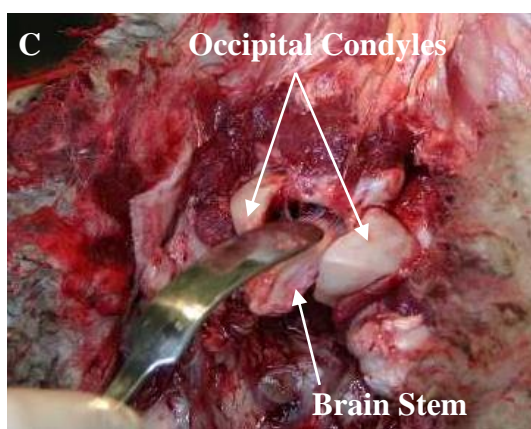
The brainstem cannot be severed if the spoon is inserted incorrectly. There is no cutting surface available to sever the sample. The tip of the spoon pushes into the mid brain and does not allow removal of brain stem.

**Figure 15: Sampling Fallen and Casualty Slaughter Sheep and Goats**

Prionics sampling involves the removal of the brain stem from the head using a specially modified spoon called a 'prionics spoon' as seen in Figure A.



After removing the head, hold it with the rostral (nose) end pointing down as in Figure B. If necessary, clear away any excess muscle or fat from the occipital condyles to allow clear access to the foramen magnum and brain stem.



Aim to slide the prionics spoon through the occipital condyles and over the dorsal surface of the brain stem. Removal of dura mater is not necessary however connective tissue surrounding the brain stem can be broken with the index finger.

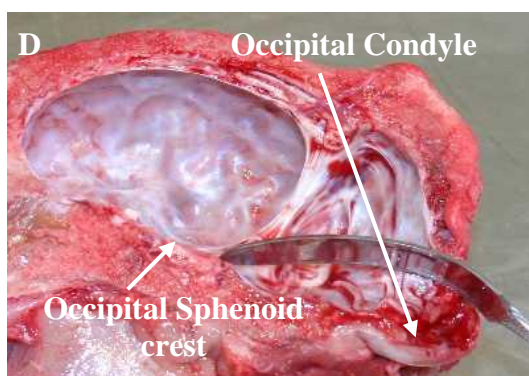
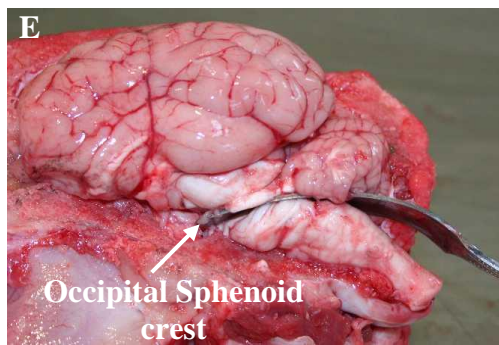


Figure D shows an example of the final placement of the spoon minus the brain. Note the tip firmly touching the occipital-sphenoid crest and the spoon forming an arc from the occipital condyles down to the occipital-sphenoid crest.



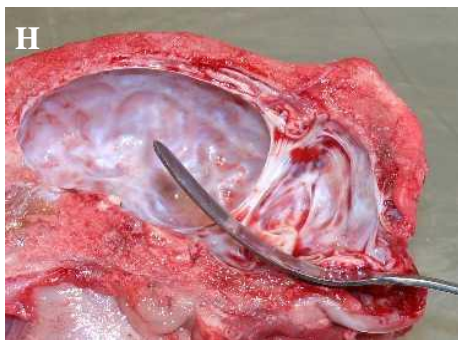
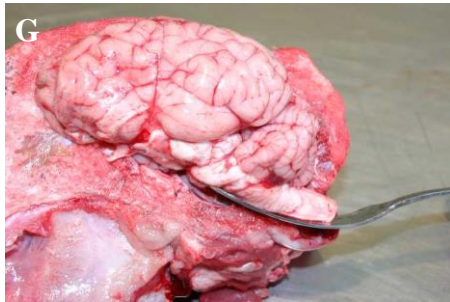


Once the spoon is in the foramen magnum, it is important to keep the tip of the spoon pointing dorsally until  $\frac{3}{4}$  of the blade is past the occipital condyles. Then, push the blade ventrally and firmly against the occipital-sphenoid crest. Ensure the tip remains caudal to the crest (Figure E) to provide a cutting surface for the spoon. Move the tip of the spoon from side to side to insure a proper cut.



The brain stem should be made up of a small section of the spinal cord, obex and part of the medulla and should be placed in a yellow top container, details recorded on the side and chilled to 4°C, or frozen to less than -20°C as soon as possible if receipt at the primary testing laboratory is expected to be longer than 3 days from the death of the animal.

### INCORRECT TECHNIQUE



**INCORRECT TECHNIQUE** - The brain stem cannot be severed if the spoon is inserted incorrectly. Figures G and H show an example of the spoon being used upside down. There is no cutting surface available to sever the brain stem. The tip of the spoon pushes into the mid brain and does not allow removal of brain stem.

## **GUIDELINES FOR PROVISION OF FINANCIAL INCENTIVES - FALLEN AND CASUALTY SLAUGHTER**

Claims for payment of costs associated with sample submission and testing should be sent to Animal Health Australia, preferably within one month of the end of each quarter.

### **Australian Centre for Disease Preparedness**

Payments are available for laboratory testing eligible fallen or casualty slaughter samples.

- Bio-Rad testing - \$129 per sample

### **Department of Agriculture, Fisheries and Forestry**

Payment of \$15 per sample (plus GST) is available to DAFF for the collection of samples from eligible fallen or casualty stock.

Freight will be charged directly to AHA as a special freight service has been arranged for the shipping of these samples.

## APPENDIX 4 - CLINICAL HISTORY AND POST-MORTEM REPORT

Date examined \_\_\_\_\_

Owner/manager \_\_\_\_\_

PIC \_\_\_\_\_

Property Address \_\_\_\_\_

NLIS/RFID Number \_\_\_\_\_

Animal type  
(please circle)

BOVINE or OVINE or CAPRINE

Age \_\_\_\_\_ estimate in months or years

Enterprise Type  
(please circle)

Meat or Milk or

Fibre or Feedlot

Imported Animal?  
(please circle)

YES or NO

Home bred?  
(please circle)

YES or NO

Clinical history including treatment (if administered) and post mortem findings

Provisional diagnosis

What samples have been submitted?

- ☐ Unfixed, frozen cervical spinal cord (2 – 3 cm)
- ☐ Fresh dorsal third of cerebellum (sheep & goats)
- ☐ Whole, undistorted brain (preferably fixed)
- ☐ Other tissue specimens (optional) – recommended to support alternate diagnosis

Tick minimum of two (2) neurological and behavioural changes consistent with BSE or scrapie shown by this case

## Mental Status

- ☐ Altered consciousness
- ☐ Apprehension
- ☐ Behaviour change
- ☐ Excitability
- ☐ Frenzy
- ☐ Hesitation at doors, gates, barriers
- ☐ Herd hierarchy change
- ☐ Moribund (without infection/trauma)
- ☐ Teeth grinding
- ☐ Temperament change

## Sensation

- ☐ Blindness
- ☐ Excessive licking of nose or flank
- ☐ Head rubbing or pressing
- ☐ Head shyness
- ☐ Hyperaesthesia (sound, touch)
- ☐ Hypoaesthesia (sound, touch)
- ☐ Rubbing/Itching
- ☐ Kicking persistently when milked
- ☐ Wool loss (flank & hind quarter)

## Posture/Movement

- ☐ Abnormal ear position
- ☐ Abnormal head carriage
- ☐ Ataxia
- ☐ Circling
- ☐ Falling
- ☐ Fetlock knuckling
- ☐ Paralysis/paresis
- ☐ Recumbency
- ☐ Tremor

Name of submitter (AHO/Veterinarian) (print) \_\_\_\_\_

Business name and address (print) \_\_\_\_\_

Incentives are not paid where:

- inadequate reports or specimens are submitted
- the animal does not meet eligibility criteria <sup>9</sup>

Maximum payment = 2 animals per disease outbreak

SUBMITTER SIGNATURE

DATE

Office Use Only

DATE FORM RECEIVED

## Recommendation

- ☒ Clinically consistent animal (eligible for subsidy)
- ☒ Fallen (Dead) animal (NOT eligible for subsidy)
- ☒ Casualty (Down) slaughter animal ☐ (NOT eligible)

NTSESP COORDINATOR SIGNATURE

DATE

**Eligible cattle** are older than 30 months of age and **less than 9** years, that display at least two (2) behavioural changes or neurological signs without evidence of infectious disease.

**Eligible sheep and goats** are 18 months of age or more (but preferably not more than five years old), that display at least two (2) clinical signs compatible with scrapie.

## APPENDIX 5 - NTSESP CONTACTS

The relevant National, State or Territory coordinator below should be contacted if further details are required on the NTSESP.

Name	Organisation	e-mail
Rob Barwell	TSEFAP Manager	<a href="mailto:rbarwell@animalhealthaustralia.com.au">rbarwell@animalhealthaustralia.com.au</a>
Jenny Voss	Tas. State Coordinator	<a href="mailto:jennifer.voss@nre.tas.gov.au">jennifer.voss@nre.tas.gov.au</a>
Scott Ison	Vic. State Coordinator	<a href="mailto:scott.ison@agriculture.vic.gov.au">scott.ison@agriculture.vic.gov.au</a>
Richard Ploeg	Australian Centre for Disease Preparedness	<a href="mailto:Richard.Ploeg@csiro.au">Richard.Ploeg@csiro.au</a>
Will Janson	WA State Coordinator	<a href="mailto:Will.Janson@dpird.wa.gov.au">Will.Janson@dpird.wa.gov.au</a>
Kathleen Moylan	NT State Coordinator	<a href="mailto:kathleen.moylan@nt.gov.au">kathleen.moylan@nt.gov.au</a>
Geoff Campbell	NSW State Coordinator	<a href="mailto:geoff.campbell@dpi.nsw.gov.au">geoff.campbell@dpi.nsw.gov.au</a>
Diana Miller	SA State Coordinator	<a href="mailto:diana.miller@sa.gov.au">diana.miller@sa.gov.au</a>
Joanne Mollinger	Qld State Coordinator	<a href="mailto:Joanne.Mollinger@daf.qld.gov.au">Joanne.Mollinger@daf.qld.gov.au</a>
Christine Coulson	DAFF Coordinator	<a href="mailto:Christine.Coulson@aff.gov.au">Christine.Coulson@aff.gov.au</a>

### Website

The BSE AUSVETPLAN, the national TSE database, these guidelines, and other information on the NTSESP and TSEFAP in general can be found on the Animal Health Australia website: <http://www.animalhealthaustralia.com.au>



P 61 2 6232 5522

E [aha@animalhealthaustralia.com.au](mailto:aha@animalhealthaustralia.com.au)

[www.animalhealthaustralia.com.au](http://www.animalhealthaustralia.com.au)