

PROTOCOL FOR THE MANAGEMENT OF  
DESIGNATED ZOO ANIMALS IMPORTED  
FROM COUNTRIES AT RISK FOR  
TRANSMISSIBLE SPONGIFORM  
ENCEPHALOPATHIES (TSES)

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# 1 INTRODUCTION

Transmissible Spongiform Encephalopathies (TSEs) are nationally notifiable diseases. Australia is free from TSEs in animals, and is recognised as meeting World Organisation for Animal Health (OIE) requirements for a bovine spongiform encephalopathy (BSE) *Negligible Risk* and scrapie free country.

BSE has been detected in several countries, a current overview of which can be found on a World Organisation for Animal Health webpage at <http://www.oie.int/animal-health-in-the-world/bse-specific-data/>. Scrapie occurs in ovine and caprine species in many countries, and Chronic Wasting Disease (CWD) has been diagnosed in cervids across North America.

Live animals were imported into Australia from Japan, European Union Member States including the United Kingdom, Canada and USA before BSE was detected in these countries.

Australia operates a nationally integrated program of active surveillance for TSEs, known as the National TSE Surveillance Program (NTSESP). This is funded by industry and governments to demonstrate Australia's on-going freedom from BSE and scrapie in domestic ruminants and to provide early detection of these diseases if they occur. The objective of the NTSESP is to support trade by maintaining a surveillance system which is consistent with the OIE Terrestrial Animal Health Code, and to assure all importing countries that Australia remains free of BSE and scrapie.

The diagnosis of spongiform encephalopathy in an imported Golden Cat that died at Melbourne Zoo (2002) demonstrated that TSE surveillance needed to be extended to certain imported zoo animals. In addition a nationally uniform approach to the management of these zoo animals is appropriate.

This protocol documents a national approach to management of risk-animals and the response to a positive TSE diagnosis in designated animals within the Australian zoo population. The first version was prepared by an Animal Health Committee Working Group in July 2003.

## 2 ANIMALS OF INTEREST

This protocol applies to the following animals, hereinafter referred to as "designated zoo animals" that meet the following definition:

A designated zoo animal is a mammal, meeting the following definition, living within a registered zoo or wildlife park in Australia.

### 1.1 BSE

a) All felidae, bovidae and primates having lived any part of their life in a country not listed as having a negligible or controlled BSE status by the OIE (see <http://www.oie.int/?id=495>) **and** having spent less than 15 years continuously in Australia following their most recent arrival

OR

b) All felidae, bovidae and primates having spent less than 15 years continuously in Australia following their most recent arrival and having lived in the following countries before 2001: Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Israel, Italy, Japan, Liechtenstein, Luxembourg, the Netherlands, Poland, Portugal, Slovakia, Slovenia, Spain, Switzerland and the UK, or any other country listed as having the OIE controlled BSE status (see <http://www.oie.int/?id=495>).

### 1.2 Chronic Wasting Disease (CWD)

All cervidae having spent any portion of their life in USA and Canada, or any other country in which CWD has been reported.

### 1.3 Scrapie

All members of the genera Ovis and Capra, imported from any country except New Zealand.

Note:

1) TSEs should be a differential diagnosis for any susceptible species showing indicative clinical signs, regardless of being imported or not, and relevant sampling should be undertaken.

2) It is recommended that managers of private animal collections, which are not registered zoos or wildlife parks, should also follow the guidelines that are contained in this protocol.

## **3 MANAGEMENT OF DESIGNATED ZOO ANIMALS**

With the exception of scrapie and CWD, TSEs affecting zoo animals are not contagious, and provided affected animals are kept out of the human and animal food chain, there is negligible risk of spread to in-contact animals and cohorts, or contamination of the environment. Specific management measures are not required for progeny or in-contact animals, or animal enclosures. Management of designated zoo animals while they are alive involves minimal special procedures, with the main focus being applied to diagnostic and disposal measures when such animals die or are euthanized.

### **3.1 SURGERY**

Normal standards of aseptic surgical practice and associated Work Health & Safety (WH&S) procedures are adequate to address the negligible risks associated with the conduct of surgical procedures on designated zoo animals.

### **3.2 COLLECTION AND EXAMINATION OF BLOOD AND TISSUE SAMPLES**

Contemporary standards of operation of pathology laboratories satisfactorily address the very low risk associated with diagnostic examination of blood and tissue samples.

No intervention above normal practice is required. Blood and tissue samples should only be forwarded to laboratories operating to contemporary standards and with waste disposal arrangements meeting regulatory requirements for medical waste.

### **3.3 STORAGE OF TISSUE SAMPLES**

It is common practice for zoos to store a range of blood and tissue samples including semen, blood cell masses, reproductive material, formalin-fixed and fresh-frozen tissues and other material.

This material needs no special handling, storage or treatment in the case of designated zoo animals, although correlation and identification of stored samples from the donor animal should be routinely undertaken.

Should a case of TSE be diagnosed in a designated zoo animal, refer to section 4.3 for requirements for storage of tissue samples.

### **3.4 BITE AND NEEDLE STICK TYPE INJURIES ASSOCIATED WITH HANDLING OF DESIGNATED ZOO ANIMALS**

There is no evidence that personnel handling designated zoo animals are at any increased risk with respect to personal health and safety.

Normal first aid and related WH&S procedures should be applied.

### **3.5 POST-MORTEM EXAMINATION OF, AND THE COLLECTION AND SUBMISSION OF SAMPLES FROM, DESIGNATED ZOO ANIMALS**

As a general principle, all designated zoo animals should receive a complete post mortem examination irrespective of the clinical presentation of the animal prior to death.

Designated zoo animals that have died or been euthanized constitute eligible cases for inclusion in the National TSE Surveillance Program (NTSESP). Accordingly, post mortem examination for evidence of TSEs is required in each case<sup>1</sup>. This will also provide information of scientific interest and provide useful cases for diagnostic practice. To facilitate this, it is recommended that zoo animal records and inventories be marked “Designated zoo animal – necropsy and laboratory testing of samples for TSE on death or euthanasia”.

Annex 1 details guidelines for performing post mortem examinations and sample collections from designated zoo animals for the purpose of TSE exclusion.

The State/Territory CVO must be notified by the zoo veterinarian of the death of a designated zoo animal, and the actions being taken in accordance with this protocol.

State NTSESP coordinators are a useful contact for zoo veterinarians who may not be familiar with brain removal and submission procedures. A list of NTSESP coordinators is attached at Annex 3.

### 3.6 DISPOSAL OF DEAD ANIMALS

The carcass of any designated zoo animal must be disposed of in a manner that prevents any tissue material entering the animal or human food chain. Normal medical waste disposal arrangements are satisfactory as these involve incineration. Incineration is preferred but deep burial is acceptable. Rendering into meat and bone meal is not an acceptable means of disposal.

As an important general rule, no carcass of **any** designated zoo animal should be used as food for other animals in the zoo collection, unless the risk of transmissible diseases has been assessed by a veterinarian.

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<sup>1</sup> The costs associated with testing will be borne by the state departments for ovine and bovine samples.

## 4 RESPONSE ACTION TO A TSE DIAGNOSIS

The following is applicable to a **BSE-related** case being diagnosed in a zoo animal. It is based on the understanding that the carcasses of zoo animals in Australia are appropriately disposed of, without access by zoo carnivores or otherwise entering the animal or human food chain, in accordance with 3.6 above.

Provided the protocol for the post-mortem examination of a designated zoo animal (*refer to Annex 1*) has been followed, the positive diagnosis of a **BSE-related** case will generally require minimal response action.

Upon notification of a positive result by Australian Animal Health Laboratories (AAHL), the State/territory CVO will notify the Chair of Consultative Committee for Emergency Animal Disease<sup>2</sup> (CCEAD).

Unless otherwise determined by CCEAD the following is to apply:

### 4.1 PROGENY AND IN-CONTACT ANIMALS

There is negligible risk of transmission through contact with infected live animals. No specific management measures are necessary for in-contact animals or progeny of designated zoo animals. Routine records should allow any progeny to be identified and their location determined for future reference.

### 4.2 ANIMAL ENCLOSURES

There is negligible risk of spread into the environment through live animals. No specific management measures are necessary for cleaning the animal enclosure in which the affected animal lived.

### 4.3 STORED TISSUE MATERIAL

Conditions for storage, handling and disposal of stored tissue material from BSE-positive animals should be discussed with the State/Territory CVO. Any stored material from the positive animal should be flagged as such. Discarding of the material would not be necessary until or unless it becomes of no continuing use. Unless otherwise determined by CCEAD, no restrictions apply to using stored reproductive material from these animals. Researchers or other third parties seeking access to stored material should be advised by the zoo that the material derives from an animal diagnosed with a TSE condition.

## OTHER TSES

In the case of a confirmed diagnosis of scrapie or CWD in a zoo animal, response actions will involve specific consideration of in-contact animals and the environment, as both diseases are capable of

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<sup>2</sup> CCEAD is a coordinating body providing the technical link between the Commonwealth, states, territories and industry for decision making during animal health emergencies.

vertical and lateral transmission. These response measures will be in accordance with AUSVETPLAN, as determined by CCEAD taking into account the particular circumstances.



## ANNEX 1

### Guidelines for performing post mortem examinations and sample collections from designated zoo animals for the purpose of TSE exclusion

#### 1. Background

- Transmissible spongiform encephalopathies (TSEs) are fatal degenerative brain diseases affecting a variety of species including humans (variant Creutzfeldt-Jakob Disease - vCJD), domestic ruminants (Bovine spongiform encephalopathy – BSE, scrapie), domestic cats (feline spongiform encephalopathy – FSE), farmed and rangeland cervidae (Chronic Wasting Disease - CWD), and farmed mink (transmissible mink encephalopathy), as well as several species of zoo ungulates (exotic ungulate spongiform encephalopathy), non-human primates and felidae.
- Molecular and biological investigations have supported an etiological link between BSE and vCJD, FSE, exotic ungulate spongiform encephalopathy and people.
- The causative agents of TSEs, termed ‘prions’, are not uniformly distributed in the tissues of affected individuals and infectivity depends upon the stage of incubation. In general, late in the incubation period and during clinical disease neural tissues (including the eye) have the highest concentration of prions, followed by spinal fluid and lymphoid tissues.
- Prions exhibit unusual resistance to autolysis as well as common chemical and physical decontamination methods including autoclaving at conventional times and temperatures (121°C for 15 min).
- Transmission of scrapie, CWD, FSE, or exotic ungulate spongiform encephalopathy to humans has not been recognised. If transmission did occur in an occupational setting this would most likely be from exposure to infected tissues or materials by direct inoculation, splashing of mucous membranes, or inadvertent ingestion. Transmission is unlikely to occur through the aerosol route. Transmission of BSE to humans, leading to vCJD, by any means other than ingestion of infected tissue has not been recognised.
- Although there has been no recognised transmission other than by ingestion, special consideration should be given to the protection of involved personnel from possible exposure to prions during performance of post mortem examinations and sample collections from designated zoo animals for the purpose of TSE exclusion. Appropriate decontamination of tools, instruments and contaminated protective clothing should be considered. The appropriate containment of environmental contaminants should also be considered.
- As a general principle, all designated zoo animals should receive a complete post mortem examination irrespective of the clinical presentation of the animal prior to death.

#### 2. Protection of personnel from possible exposure to prions during post mortem examinations and sample collections from designated zoo animals for the purpose of TSE exclusion

- Only personnel approved by the institution and directly involved in the procedure should be present.

- Basic hygiene precautions are applicable:
  - Do not eat, drink, smoke or take medication while performing or witnessing the post-mortem examination
  - Protect skin wounds prior to starting the post mortem examination
  - Wear protective clothing including Wellington boots and disposable gloves. Consideration should be given to use of disposable impervious back-fastening gowns or aprons and waterproof leggings
  - Protect eyes and mucous membranes – safety spectacles and (N95/P2) masks or full face visor as appropriate and including consideration of operator preference (see Annex 2). Reference should be made to Australian Standard AS/NZS 1715 - Selection, use and maintenance of respiratory protective devices
  - Minimise the use of sharps and where possible use single-use disposable items e.g. scalpels
  - Use of suitable hand protection is advised, for example armoured or cut resistant gloves, especially during collection of the brain or spinal cord (see Annex 2). It should be emphasised that such gloves do not protect against needle-stick or other penetrating injuries
  - Potential generation of bone dust contaminated with neural tissue will be less if hacksaws or bone forceps are used, rather than reciprocating or oscillating saws, for removal of the brain or spinal cord
  - Remove protective clothing and wash hands before leaving the work area
  - Accidents involving parenteral exposure to tissues or contaminated materials should be documented. It has been advised that such records be retained for 40 years (UK Advisory Committee on Dangerous Pathogens, <http://www.hse.gov.uk/aboutus/meetings/committees/acdp/>).

### **3. Decontamination and/or disposal of tools, instruments and contaminated waste**

- Single use sharps (in approved containers) and contaminated waste including disposable gloves, aprons, polythene or other sheeting, as well as the carcase itself should be secured in leak proof containers, e.g. double bagged, and disposed of safely by incineration.
- Tools, instruments, and work surfaces intended for reuse during subsequent post mortem examinations should be washed thoroughly with detergent to remove adherent material prior to decontamination. For preference, cleansing should be carried out in an automated thermal washer, with liquid waste disposed of by normal direct discharge or by collection and inactivation, rather than by manual handling. Subsequent decontamination is by the following as appropriate
  - sodium hypochlorite solution containing 2% (20000ppm) available chlorine for more than one hour at 20<sup>o</sup> C for surfaces (overnight for equipment). It should be noted that the strength of commercial hypochlorite may vary during storage

or

- 80g/L sodium hydroxide for more than one hour at 20<sup>0</sup> C for surfaces (overnight for equipment)

or

- porous load autoclaving at a hold temperature of 134-138<sup>0</sup> C for a single cycle of 18 minutes at 30lb/in<sup>2</sup> or six successive cycles of three minutes each or at 132<sup>0</sup> C for 270 minutes.

Depending upon the circumstances, cleaning and decontamination may be carried out simultaneously e.g. cleaning with sodium hydroxide.

Note: Use of a washing machine to wash protective clothing will result in contamination of the machine. To avoid this, clothing can be sealed and frozen until results of testing are available. If negative, clothes can be washed as normal. If positive, clothes and other items should be double bagged and incinerated.

#### **4. Containment of environmental contamination**

- Prior to commencement of the work, consideration should be given to ease of decontamination of the working surfaces following completion of the post mortem examination. For example, work may be carried out on a stainless steel or enamel tray that may subsequently be autoclaved or decontaminated using appropriate disinfectant (see above). Work surfaces may also be protected by disposable coverings such as polythene sheeting or plastic-backed absorbent paper.
- All instruments, tools, specimen jars, etc. should be assembled prior to commencing the post mortem to minimise traffic between 'clean' and 'dirty' work areas. A 'clean' assistant should be available to record observations, label specimen tubes etc.
- Washing down of the work area should be minimised. Consideration may be given to collection of washings in a sump or tank for decontamination (see above) prior to disposal.

#### **5. Specimens required for TSE exclusion**

- Whole intact brain should be collected and fixed in 10% neutral buffered formalin for histological examination. Submission of the entire brain to the laboratory facilitates provision of information that might contribute to differential diagnosis and reduces the opportunity that a TSE case might be missed.
- Cervical spinal cord segment(s), retrieved from the exposed cranial surface of the atlas following removal of the head, stored *without fixation* at -20<sup>0</sup> C or below for further testing where indicated.
- Representative samples of lymphoid tissues including spleen, tonsil, Peyer's patches and lymph nodes fixed in 10% neutral buffered formalin and also stored frozen without fixation (as above).

## 6. Transport of specimens

When transporting specimens the following advisory needs to be observed.

Formaldehyde solutions containing less than 25%, but 10% or more formaldehyde must be shipped under the Proper Shipping Name '**Aviation regulated liquid, n.o.s.**' (Class 9 miscellaneous Dangerous Goods)<sup>3</sup>




**NB:** Refer to the National TSE Surveillance Project (NTSESP) National Guidelines for Field Operations for further detail on sampling requirements

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<sup>3</sup> IATA Infectious Substances and Diagnostic Specimens Shipping Guidelines 2003. 3.3.7 Other dangerous goods (Page 40)

**ANNEX 2**

**Safety equipment that may be suggested for use with post mortem examinations from designated zoo animals for TSE exclusion**

Apron	
Over-pants Rubber	
Kevlar cut resistant gloves	
<p>Tyvec overall</p> <p>Coveralls disposable Tyvek</p> <p>Overalls Kleenguard®</p>	
<p>RBF (Reusable Barrier Fabric) theatre gowns, suits, footwear, headwear, drapes, wraps etc., all with excellent fluid resistance</p>	

### ANNEX 3

#### NATIONAL TSE SURVEILLANCE PROJECT – STATE AND AHA COORDINATORS

Name	Address	Phone/Fax/Email
Dr Robert Barwell	Animal Health Australia PO Box 5116, Braddon, ACT 2612	P 02 6203 3947 F E <a href="mailto:rbarwell@animalhealthaustralia.com.au">rbarwell@animalhealthaustralia.com.au</a>
Dr Emma Watkins	Department of Primary Industry, Parks, Water and Environment GPO Box 44, Hobart TAS 7001	P 03 6165 3264 F E <a href="mailto:emma.watkins@dpiwve.tas.gov.au">emma.watkins@dpiwve.tas.gov.au</a>
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## ANNEX 4

### QUICK REFERENCE GUIDE

#### ACTIONS REQUIRED IN THE EVENT OF DEATH OF A TSE DESIGNATED ZOO ANIMAL

**ALL designated zoo animals should receive a complete post mortem examination to determine the cause of death.** These guidelines have been developed to ensure investigation is conducted in a nationally consistent manner.

#### Administration

*(Note: zoos should, in advance, fill in the relevant information below, for their jurisdiction, to allow quick access when required)*

1. National Transmissible Spongiform Encephalopathies Surveillance Project state/territory coordinators may be good sources of information. See Annex 3

Name: \_\_\_\_\_ Contact details: \_\_\_\_\_

2. The state/territory CVO must be informed of the death.

Name: \_\_\_\_\_ Contact details: \_\_\_\_\_

#### Necropsy - preparation

1. For human health and safety reasons, the number of staff involved (both directly and indirectly) in the post mortem examination should be minimised. Nevertheless, the staff member in charge of the post mortem procedure should ensure sufficient personnel are present to safely manage all aspects of necropsy and manual handling.
2. One person should be assigned as a clean scribe/runner, to minimise contamination risks.
3. Minimum Personal Protective Equipment (PPE) to be worn during the post mortem examination:
  1. Disposable, impermeable gloves and
  2. N95/P2 mask and
  3. Eye protection (against splash) and
  4. Wellington boots (gumboots) and
  5. Protective clothing which can be removed prior to leaving necropsy area and
  6. All open wounds must be covered with a waterproof dressing.
4. Consideration should also be given to using the following, higher level PPE:
  1. Disposable, impermeable gowns or coveralls (note: impermeable coveralls can quickly result in bodily overheating in a warm environment or when staff are undertaking physical exertion. It is recommended that they be worn for a maximum of 20 minutes at a time in conditions of heat stress).
  2. Cut resistant gloves (EN 388)
5. Consideration should be given to ease of decontamination of the working surfaces at completion of the post mortem examination. Washing down of the work area should be minimised and consideration may be given to collection of washings in a sump for decontamination prior to disposal.
6. In the case of large carcasses, the necropsy should preferably be undertaken in a suitable enclosed area which can be appropriately disinfected at the end of the

procedure. Performing necropsies outdoors may mean that effective disinfection of the environment is difficult to achieve, due to organic material such as soil and vegetation. If possible, outdoor necropsies should be performed in areas and on surfaces that can be effectively disinfected. Alternatively, contamination risk may be minimised if removal of the brain and examination of the gastro-intestinal tract are undertaken in a contained, indoor environment. Consideration may also be given to arranging for the State/ Territory veterinary laboratories (which would have suitable indoor facilities for large animal necropsy) to perform the necropsy.

### **Necropsy – sample collection**

*Note: The brain, spleen and gut-associated-lymphoid tissue (GALT) are known foci of prion proliferation and should be sampled as priorities. The GALT include enteric lymph nodes, tonsils, Peyer's patches and retropharyngeal lymph nodes. If there is difficulty in identifying some of these less frequently sampled tissues, collection of brain, spleen and enteric lymph nodes will be sufficient.*

7. A representative sample (ideally  $\geq 1 \text{ cm}^3$ ) of the following tissues should be collected and stored frozen, preferably at  $-80^\circ \text{C}$ 
  1. Cervical spinal cord (collected from cranial surface of atlas following removal of head)
  2. Lymph nodes (a representative sample of 4- 6 nodes from throughout the body, including if possible third eyelid, mesenteric lymph node and retropharyngeal lymph node)
  3. Spleen
  4. Tonsils (the easiest to locate may be palatine)
  5. Peyer's patches (found on the antimesenteric surface of the ileum)
8. Collect the following tissues fixed in 10% neutral buffered formalin. Sections should be no greater than 1cm thick in one plane.
  1. Whole brain with brain stem attached, immersion fixed as one whole organ for a minimum of three days, preferably a week, in a large container and with sufficient volume of formalin to allow brain to float freely, **cerebellum facing downwards**
  2. Lymph nodes
  3. Spleen
  4. Tonsils
  5. Peyer's patches

### **Clean up**

1. Carcase and disposable materials should be double bagged and incinerated.
2. Carcass disposal by deep burial is also acceptable. Rendering into meat and bone meal is not an acceptable means of disposal.
3. Tools, instruments, surfaces and protective clothing should be cleaned of visible material with detergent and then decontaminated using sodium hypochlorite, sodium hydroxide or autoclaving (see Annex 1 for details).

*Note: Use of a washing machine to wash protective clothing will result in contamination of the machine. To avoid this, clothing can be sealed and frozen until results of testing are available. If negative, clothes can be washed as per normal. If positive, clothes and other items should be double bagged and incinerated.*

See also: <http://www.animalhealthaustralia.com.au/what-we-do/disease-surveillance/tse-freedom-assurance-program/surveillance-of-tses/> for more details on post mortem, sample collection and processing details.





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