Australian Government
Department of Agriculture, Fisheries and Forestry

# Guidelines for managing the risk of transmitting transmissible spongiform encephalopathies (TSEs) via veterinary vaccines and other in vivo veterinary products

**October 2012**

Post: GPO Box 858 (18 Marcus Clarke St) Canberra ACT 2601 Location: 7 London Circuit Tel: +61 2 6272 3933 www.daff.gov.au

© Commonwealth of Australia

**Ownership of intellectual property rights**
Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Commonwealth of Australia (referred to as the Commonwealth).

**Creative Commons licence**
All material in this publication is licensed under a Creative Commons Attribution 3.0 Australia Licence, save for content supplied by third parties, photographic images, logos, and the Commonwealth Coat of Arms.

Creative Commons Attribution 3.0 Australia Licence is a standard form licence agreement that allows you to copy, distribute, transmit and adapt this publication provided that you attribute the work. A summary of the licence terms is available from creativecommons.org/licenses/by/3.0/au/deed.en. The full licence terms are available from creativecommons.org/licenses/by/3.0/au/legalcode.
This publication (and any material sourced from it) should be attributed as:

> Department of Agriculture, Fisheries and Forestry (2012) *Guidelines for managing the risk of transmitting transmissible spongiform encephalopathies (TSEs) via veterinary vaccines and other in vivo veterinary products*. Department of Agriculture, Fisheries and Forestry, Canberra. CC BY 3.0.

**Internet**
*Guidelines for managing the risk of transmitting transmissible spongiform encephalopathies (TSEs) via veterinary vaccines and other in vivo veterinary products* is available via daff.gov.au.

**Disclaimer**
The Australian Government acting through the Department of Agriculture, Fisheries and Forestry has exercised due care and skill in the preparation and compilation of the information in this publication. Notwithstanding, the Department of Agriculture, Fisheries and Forestry, its employees and advisers disclaim all liability, including liability for negligence, for any loss, damage, injury, expense or cost incurred by any person as a result of accessing, using or relying upon any of the information in this publication to the maximum extent permitted by law.

**Acknowledgement**
DAFF acknowledges Dr Peter Hewitt as the primary author of this document.

## CONTENTS

## ACRONYMS AND GLOSSARY

| | |
|---|---|
| amyloid | extracellular, proteinaceous deposit exhibiting cross-beta structure due to misfolding of unstable proteins |
| atypical BSE | a BSE case that does not follow the typical pattern of classic BSE disease |
| BSE | bovine spongiform encephalopathy |
| buffy coat | the white blood cell containing fraction of anticoagulated blood after centrifugation. It sits on top of the red blood cell fraction and below the plasma |
| CJD | Creutzfeldt-Jakob disease |
| CWD | chronic wasting disease of deer and elk<br>a TSE of North American deer and elk, a progressive neurodegenerative disorder that produces spongiform changes in the brain and chronic weight loss leading to the death of these animals. There is no known relationship between CWD and any other TSE of animals or people |
| DAFF | Australian Government Department of Agriculture, Fisheries and Forestry |
| downer animal | non-ambulatory animal, generally defined as livestock that cannot rise from a recumbent position or that cannot walk |
| EFSA | European Food Safety Authority |
| ELISA | enzyme-linked immunosorbent assay<br>a process in which brain tissue is homogenized, normal prion protein is destroyed by a protease enzyme, and any remaining abnormal prion protein is bound to the surface of a clear, microtiter well. Abnormal proteins are detected immunologically using antibodies linked to an enzyme and exposed to a chemical substrate, which provides a signal in the form of a color change or light emission. An electronic instrument called a spectrophotometer picks up the signal. In the United States, non-negative test results for TSE using the ELISA method are confirmed through immunohistochemistry and western blot testing. ELISA tests for TSE typically can be completed in about four hours |
| EMA/EMEA | European Medicines Agency (formally EMEA: European Medicines Evaluation Agency) |
| encephalopathy | any disease in which the functioning of the brain is affected without the brain showing an inflammatory response |

| | |
|---|---|
| FBS | foetal bovine serum |
| FSE | feline spongiform encephalopathy believed to be caused by the ingestion of the BSE agent |
| ileum | the lowest part of the small intestine, located beyond the duodenum and jejunum, just before the large intestine (the colon) |
| immunoblot/Western blot | a biochemical technique in which brain tissue is homogenized and treated with a protease enzyme that destroys normal prion protein but not the abnormal protein. The brain sample is separated by gel electrophoresis (a method that separates protein molecules based on size, electric charge and other physical properties). Abnormal prion protein molecules can be detected using antibodies linked to an enzyme that results in a chemical reaction (staining) |
| immunohistochemistry | a laboratory method that involves microscopic examination of brain tissue that has been reacted with antibodies for proteins. The antibodies are linked to enzymes that show a chemical reaction (called staining) that can detect the abnormal form of prion protein found in TSE |
| *in vitro* | in an artificial environment outside the living organism (e.g. in the laboratory) |
| *in vivo* | within a living organism |
| incidence | the frequency with which something, such as a disease, appears in a particular population or area. In disease epidemiology, the incidence is the number of newly diagnosed cases during a specific time period. The incidence is distinct from the prevalence which refers to the number of cases alive on a certain date |
| indigenous BSE case | non-imported BSE case |
| isoform | any of the three forms of the abnormal prion protein as distinguished in western blots: unglycosylated, mono-glycosylated or di-glycosylated isoforms |
| leucodepletion | the removal of white blood cells |
| MSB | master seed bacteria |
| MSV | master seed virus |
| neural | having to do with nerve cells |
| OIE | World Organisation for Animal Health |
| OIE Code | World Organisation for Animal Health *Terrestrial Animal Health Code* |

| | |
|---|---|
| peripheral nerves | nerves that lie outside of the brain and spinal cord |
| pithing | the laceration of central nervous tissue by means of an elongated rod-shaped instrument introduced into the cranial cavity of slaughter cattle after stunning |
| prion | infectious agent causing TSE. A prion is a protein that occurs normally in a harmless form. By folding into an aberrant shape, the normal prion turns into a rogue agent. It then co-opts other normal prions to become rogue prions |
| probiotics | live microorganisms which, when administered in adequate amounts, confer a health benefit on the host |
| PrP | prion protein, encoded by the gene PRNP, expressed by many cell types and many organisms |
| $PrP^{BSE}$ | resistant prion protein associated with bovine spongiform encephalopathy; also called $PrP^{Sc}$ |
| $PrP^{C}$ | naturally occurring cellular prion protein found in cells of the central nervous system (the brain and the spinal cord) and other tissues |
| $PrP^{Sc}$ | resistant prion protein associated with transmissible spongiform encephalopathies, including BSE. When the $PrP^{C}$ molecule refolds into an aberrant shape it becomes pathogenic. The disease-causing form of the normally occurring prion protein is designated as protease resistant protein ($PrP^{Res}$) or PrP Scrapie ($PrP^{Sc}$) |
| rapid test | test systems using immunological assays that detect the presence of infectious agents in animal tissues or other materials within hours |
| recombinant | recombination involves the exchange of genetic material and bringing together DNA from different sources to create a novel sequence. Proteins resulting from the expression of recombinant DNA are ‘recombinant proteins’ |
| saponification | chemical reaction which uses animal or vegetable fats mixed with alkali to produce soap |
| spongiform | resembling a sponge in being soft and full of cavities, as in transmissible spongiform encephalopathy |
| specified risk materials | also referred to as BSE risk materials and are animal tissues most likely to contain TSE infective material |
| TGA | Therapeutic Goods Administration. An agency within the Australian Government Department of Health and Ageing |
| TME | transmissible mink encephalopathy |
| trans-esterification | conversion of one ester into another via exchange of an organic group with an alcohol |

| | |
|---|---|
| TSE | transmissible spongiform encephalopathy |
| vCJD | variant (or new variant) Creutzfeldt-Jakob disease |
| western blot | a technique in molecular biology used to separate and identify proteins |
| WHO | World Health Organization |

**Glossary sources**
http://www.fao.org/docrep/010/a0999e/a0999e00.htm
http://www.medicinenet.com/mad_cow_disease/glossary.htm
http://www.bseinfo.org/glossary.aspx

## SUMMARY

Veterinary vaccines, other veterinary therapeutics and *in vivo* veterinary products play an integral role in Australian livestock production. Most in vivo veterinary products used in Australia are produced, either directly or indirectly, using at least some materials of animal origin from countries which have reported cases of transmissible spongiform encephalopathies (TSEs). It is imperative, therefore, that Australia's import policy continues to facilitate the supply of essential in vivo veterinary products, while ensuring Australia's TSE status is not compromised by potential contaminants.

Following a review of import policies for veterinary vaccines and other in vivo veterinary products, the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) issued the *Measures to address additional TSE concerns with veterinary vaccines and other high risk biologicals* in February 2002. The document was reviewed in 2004–05 in light of reports of bovine spongiform encephalopathy (BSE) in Japan and North America, and further developments in TSE research. The final recommendations, as endorsed by key stakeholders in 2005, have been used in the assessment of import permit applications, case-by-case, since then.

This review details the biosecurity requirements for various raw materials used in veterinary therapeutic and vaccine production and products derived from them. These requirements are based on a matrix of factors such as tissue or country TSE risk categories, susceptibility of the species of origin and of the target species, manufacturing method and degree of processing, and method of administration of the veterinary vaccine/therapeutic. Other factors such as age of the source animal, slaughter method, testing and TSE agent dilution may also be considered where appropriate.

Wherever possible, the use of animal-derived substrates should be avoided to minimise the risk of contamination with extraneous animal pathogens and TSE agents. If such use cannot be avoided, the raw ingredients should be sourced from countries free from diseases of quarantine concern, or otherwise tested or processed to a level to generate sufficient confidence in freedom from contamination. However, TSE agents are particularly resistant to inactivation and *in vitro* tests are generally unreliable in detecting the agent unless in neural tissue from clinical cases.

Adjustments to the guidelines may be necessary from time to time if further scientific information becomes available, international standards evolve and the number of countries reporting TSEs changes. DAFF will continue to monitor developments closely and, if necessary, will make the necessary changes to ensure the supply of essential veterinary vaccines and other therapeutics continues while avoiding compromise of Australia's TSE status. Stakeholders will be consulted on major changes. Recent amendments to the 2005 guidelines have been made to address the following developments:

1. The World Organisation for Animal Health (OIE) has amended its country classification system from five risk categories to a three risk category system (negligible, controlled and undetermined BSE risk country).

2. The World Health Organisation, the European Medicines Agency and the Therapeutic Goods Administration guidelines on TSEs have revised their tissue infectivity distribution from a four tissue infectivity category system to a three-category system (i.e. high, lower and no detectable infectivity). Modifications have also been made to address recent findings on tissue tropism of bone marrow, sheep milk and blood.

3. Atypical scrapie has been reported in Australia, Europe, New Zealand and the United States. The exposure factors, genotype distributions, tissue tropism and other epidemiological factors between atypical scrapie and classical scrapie are substantially different. TSE restrictions in relation to atypical scrapie will be applied to neural material. The previous requirements for use of other sheep tissues (i.e. non-neural) are considered adequate to address atypical scrapie.

4. Around 40 atypical BSE cases have been reported in Canada, Europe, Japan and the United States. The origin of atypical BSE remains undetermined. However, the requirements specific to BSE are considered adequate to also address any risks associated with atypical BSE. Therefore no changes specific to atypical BSE are considered necessary, other than to identify and provide a brief summary of it as a TSE.

5. Further research has confirmed the relative safety of bovine milk and milk products. The requirements for bovine milk have been amended to allow its use from both negligible and controlled risk countries.

6. Sheep and goat blood, milk and colostrum are a higher classical scrapie risk product than previously considered. Transmission of scrapie to sheep via milk and/or colostrum has been demonstrated and there appears to be greater involvement of the lymphatic system in TSE pathogenesis in sheep than in cattle. The requirements for sheep and goat milk, and blood products from classical scrapie affected countries are amended to address this increased risk.

A number of other significant changes have been made to address issues which have arisen with the previous guidelines. These include:

1. The requirements for master seed organisms and cell lines have been clarified, especially in relation to use of risk material when the master seed was created.

2. History of safe use may now be taken into consideration when determining the need to re-establish a master seed and/or the appropriate level of dilution.

3. The requirements for the use of bone-derived gelatine and collagen are better aligned with the OIE *Terrestrial Animal Health Code* which requires specified treatments for bone derived gelatine from controlled BSE-risk countries, in addition to the removal of specified risk materials.

This review will be updated periodically to take into account stakeholder comments that are supported by relevant new scientific information.

# 1 PURPOSE AND SCOPE

The purpose of this document is to review Australia's biosecurity requirements for veterinary vaccines, other veterinary therapeutics and *in vivo* veterinary products in relation to transmissible spongiform encephalopathies (TSEs).

The scope of this review includes the following biological materials that may be used in producing veterinary vaccines and other *in vivo* veterinary products:

- products derived from multiple countries or multiple species
- neural material
- milk and milk derivatives
- gelatine and collagen
- lanolin, derivatives of lanolin and wool derivatives
- lecithin (phosphatidyl choline, phosphatidyl serine, phospholipids)
- peptones and other tissue extracts
- amino acids, proteins and peptides
- tallow and tallow derivatives (e.g. glycerol, stearates)
- animal blood, serum and other blood products
- enzymes
- hormones
- cell lines
- master seed viruses, bacteria and other organisms.

For the purpose of this review, *in vivo* veterinary products include the following:

- vaccines and other veterinary immunobiologicals
- veterinary therapeutics and other pharmaceuticals
- catgut and other implantables of animal origin
- stockfeed additives
- probiotics
- other *in vivo* products (e.g. research products)
- cell lines[i]
- *in vitro* products, imported in large quantities, with the potential for diversion to *in vivo* use (e.g. bulk dehydrated culture media, bulk serum).

This review relates only to the biosecurity requirements relevant to TSEs and only for veterinary vaccines, veterinary therapeutics and *in vivo* veterinary products, and not food (for humans or animals), human therapeutics, or other products. The requirements only relate to measures to minimise the risk of transmitting TSEs via these products, and they should be strictly adhered to as any deviation would require further review and assessment by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF). All other requirements of Australia's import policies on veterinary vaccines and other veterinary products shall also be complied with. This document should be considered a working document as scientific knowledge and international standards on TSEs will continue to evolve and the number of countries reporting spongiform

[i] Cell lines are capable of being stored for many years and then may be used *in vivo* long after details of its import permit conditions (e.g. such as *in vitro* use only) are lost or forgotten.

encephalopathies may increase. DAFF will closely monitor developments and if necessary, will make adjustments to the document in consultation with operational areas and with advice to the veterinary therapeutics industry.

The review is designed to complement, and should be read in conjunction with, the current version of the following documents:

- *Australian quarantine policy and requirements for the importation of live and novel veterinary bulk and finished vaccines (1999)*
- *Specific quarantine requirements for the importation of inactivated veterinary vaccines (1997).*

It aims to be consistent with the Therapeutic Goods Administration (TGA) policy on TSEs with variation as appropriate to address specific veterinary risk factors. It also aims to be consistent, in its general principles, with the World Organisation for Animal Health (OIE) *Terrestrial animal health code* (the OIE Code) and the European Union *Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products* and its subsequent revisions by the European Medicines Evaluation Agency (EMEA; now know as the European Medicines Agency (EMA)).

The review details the biosecurity requirements for various raw materials used in veterinary therapeutic and vaccine production and products derived from them. These requirements are based on a matrix of factors including:

- tissue TSE risk category
- country TSE risk
- susceptibility of the species of origin
- susceptibility of target species
- manufacturing method and degree of processing
- method of administration of the veterinary vaccine/therapeutic
- other factors (e.g. age of the source animal, testing at slaughter and TSE agent dilution).

Unless otherwise stated, reference to the country of origin of a product refers to the country of origin of the animal from which the raw material was sourced.

## 2 INTRODUCTION

TSEs are progressive degenerative diseases and include bovine spongiform encephalopathy (BSE), feline spongiform encephalopathy (FSE), transmissible mink encephalopathy (TME), scrapie, chronic wasting disease of deer and elk (CWD) and Creutzfeldt–Jakob disease (CJD). The causative agents of TSEs are very resistant to treatment, surviving extremes of heat, radiation and chemical disinfection. Because of the long incubation period of TSEs, an infectious agent may be present in apparently healthy animals and this mandates the need for strict controls on the use of TSE risk materials in high-risk products. The dose required for transmitting TSEs by parenteral means (e.g. by injection) is generally considered to be substantially lower than by ingestion (Phillips et al. 2000). Veterinary vaccines and other biological products (biologicals), which have significant potential for direct or indirect *in vivo* veterinary use, are considered to be high-risk products.

Following a review of Australia's import policies for veterinary vaccines and other high-risk biologicals, DAFF released the document, *Measures to address additional TSE concerns with veterinary vaccines and other high risk biologicals,* in February 2002. It identified several areas

where control measures were considered essential to ensure the safety of these high-risk products. The document was reviewed in 2004-2005 in light of reports of BSE in Japan and North America, further TSE research and the release of a TSE policy by the TGA. It was released for public consultation in July 2004 and, following comments, was amended with assessments on import permit applications undertaken on a case-by-case basis. Subsequent changes to the OIE country classification on BSE, changes to internationally recognised risk categories of tissues and developments with atypical scrapie and BSE prompted further review of the document.

# 3 BACKGROUND

## 3.1 TSE agents

Prion diseases are a group of fatal neurodegenerative disorders that can present as genetic, infectious or sporadic disorders, all involving modification of the prion protein (PrP). Examples of TSEs in animals include BSE, CWD, scrapie and TME. In humans, TSEs include CJD, kuru, Gerstmann-Sträussler-Scheinker syndrome and fatal familial insomnia.

Prions are devoid of any nuclear material and are comprised entirely of protein. The normal cellular protein ($PrP^{c}$), which is characterised by a structure of four helixes (α-helixes), is converted to $PrP^{sc}$ during which two of the helixes are converted to linear structures (β-sheets). It appears that $PrP^{sc}$ acts as a template upon which the normal $PrP^{c}$ form is refolded into $PrP^{sc}$. This process may or may not be facilitated by another protein which has yet to be described (Prusiner 1998).

The incubation period of all TSEs is extremely long and variable within species; from several months in mice infected with mouse-adapted scrapie strains to three to seven years in cattle and potentially decades in humans (Collinge et al. 2006; Prusiner 1998). The ability of BSE to cross the species barrier is well demonstrated. BSE can be transmitted to cats as FSE (Pearson et al. 1991; Pearson et al. 1992) and is responsible for variant CJD (vCJD) in humans (Pocchiari 1998; Scott et al. 1999; Will et al. 1996). A case of BSE in a goat, slaughtered in France in 2002, and a possible case in the United Kingdom, initially diagnosed with scrapie in 1990, were reported in 2005 (Promed Mail 2005a; Promed Mail 2005b; Vaccari et al. 2009). Scrapie, TME and CWD have also been experimentally transmitted to cattle (Hamir et al. 2007). Considerable diversity of both TSE strains and host genotype susceptibility has been demonstrated with scrapie and CWD (Béringue et al. 2008).

Atypical scrapie, first identified in sheep in Norway and designated scrapie ‘Nor98’ (Benestad et al. 2003; Hopp et al. 2006), has since been reported in at least 13 European countries as well as in Australia, Canada, New Zealand and the United States. Knowledge of its epidemiology is still limited but, where reported, it usually appears remarkably similar in nature within and between countries. The age distribution of cases is substantially different to classical scrapie, generally affecting older sheep. The genotype distributions of atypical scrapie and classical scrapie cases are substantially different. Atypical scrapie cases do not appear to be associated with exposure factors typical of classical scrapie and it is currently thought that atypical scrapie occurs spontaneously, possibly influenced by genetic and metabolic factors. Experimental transmission via intracranial inoculation has been demonstrated. Experiments on oral transmission have not yet been completed. Atypical scrapie cases are currently only detectable by a limited number of rapid TSE detection tests due largely to the agent being more sensitive to protease compared with other TSE agents and the distribution of abnormal prion protein (Fediaevsky et al. 2008; Fediaevsky et al. 2009; Simmons et al. 2007). Atypical scrapie in a smaller number of goats has also been reported (European Commission 2008; Seuberlich et al. 2007). Chapter 14.9 of the 2011 OIE Code on scrapie

specifically ‘excludes so-called “atypical” scrapie because this condition 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’ (OIE 2011b).

Around 56 ‘atypical’ BSE cases have been reported in Canada, Europe, Japan and the United States. Two variations have been identified and termed H-type BSE and L-type BSE (also named bovine amyloidotic spongiform encephalopathy). All identified cases have been in cattle eight years of age or older. A 23-month-old animal in Japan was reported as being an L-type BSE case, but a subsequent study failed to transmit disease using tissues from this case. These atypical BSE cases differ markedly from atypical scrapie in their diagnostic characteristics, species of origin, distribution and infectivity. The origin of atypical BSE remains undetermined but possible theories include: spontaneous development, unrelated strains of prion diseases in cattle which have longer incubation periods than BSE, or that they have arisen from BSE or from TSEs in other species (TAFS 2007). One H-type BSE in Alabama in the United States has been attributed to a genetic, heritable aetiology (Richt and Hall 2008). Based on limited data from intracerebral transmission studies with transgenic mice and monkeys, L-type BSE may be more virulent with a greater attack rate and shorter incubation period in cattle and humans than classical BSE. It may also be lymphotrophic (Buschmann et al. 2006; Comoy et al. 2008; Kong et al. 2008). L-type BSE has also been transmitted orally to a macaque (EFSA Panel on Biological Hazards [BIOHAZ] 2011). In contrast, H-type BSE has failed to transmit to date to monkeys or mice transgenic for the human prion protein. Requirements of this review, as applied to BSE, have been assessed as being adequate to protect against atypical BSE.

### 3.2 TSE diagnosis and detection

Diagnosis of pre-clinical (incubating) TSEs is currently not practical. Diagnosis of clinical TSE cases is generally based on presentation of a rapidly progressive neurological disorder with myoclonus and other clinical symptoms. Diagnosis is confirmed ante-mortem by brain biopsy in humans or on post-mortem examination with the demonstration of classic histological findings of spongiform vacuolation, astrocytic proliferation, and neuronal loss (Collinge 1996). Rapid western blot and enzyme-linked immunosorbent assay tests have been refined and are being used routinely in Canada, Europe, Japan, and the United States for detecting BSE $PrP^{sc}$ in brain material obtained from targeted cattle sub-populations.

Current post-mortem tests, including the ‘rapid’ screening tests, are limited to detecting $PrP^{sc}$ in neural material taken during, or just prior to, the development of clinical signs (European Commission 1999; Knight 1998; OIE 2011; Robinson 1996). However, $PrP^{sc}$ has been detected in biopsies of palatine tonsils and nictitating membranes, using immunohistochemistry, from naturally infected sheep, genetically susceptible to scrapie, at between one-third and a half of the estimated incubation period, although no immunostaining was detected in sheep with resistant genotype (Kim et al. 2001; Schreuder et al. 1998). CWD is also detectable pre-mortem by assay of a range of peripheral tissues including rectal mucosa (Spraker et al. 2009). It can be assumed that current tests are not capable of reliably detecting the presence of $PrP^{sc}$ in substrates, finished vaccines or other products unless these contain neural material taken from clinical or near clinical animals. However, significant progress is being made with developing rapid prion detection kits for use in other tissues, including protein misfolding cyclic amplification technology (Grassi et al. 2008) and real-time quaking-induced conversion (Atarashi et al. 2011).

## 3.3 Potential TSE contamination risks

Vaccine production can involve the use of many materials of animal origin derived from various species, tissues and countries. Vaccines have the potential to expose many thousands of animals to any contaminating pathogen. The TSE risk represented by biologicals varies depending on end use, country and species of origin, processing, presentation, disposal methods and volume. *In vitro* analytical kits are generally a negligible TSE risk, whereas tissue extracts and cell lines used for production of vaccines and other *in vivo* therapeutics are potentially a high risk.

Infective agents of TSEs will survive the typical processes applied to inactivate vaccine organisms (Robinson 1996; Rutala and Weber 2001). Prion infectivity will survive treatment with formalin—often used to inactivate vaccines (Taylor 1999). Biosecurity measures applied to inactivated vaccines to manage the TSE risk should therefore be as stringent as those for live vaccines.

Contaminated vaccines, other therapeutics and other iatrogenic routes have been responsible for the transmission of TSEs:

- scrapie contaminated louping ill vaccine used in sheep in Scotland (Robinson 1996)
- scrapie contaminated *Mycoplasma agalactia* vaccine in Italy (Caramelli et al. 2001)
- CJD in humans from contaminated cadaver-derived growth hormone (Robinson 1996)
- CJD through human gonadotrophin administration, dura mater or cornea transplantation (Croes et al. 2001)
- vCJD disease or infection via transfusion of blood or a plasma product (Health Protection Agency 2009).

A more detailed account of iatrogenic transmission of TSEs can be found in the Phillips Report (Phillips et al. 2000). However, it should be noted that use of neural tissue was involved in most of the above examples and, to date, there have been no reports of the spread of BSE via vaccines.

## 3.4 TSE infectivity

The infective dose for TSEs via the parenteral route is very small and will vary with factors such as target species and route of infection. Unless derived directly from neural material, the titre in a contaminated vaccine is likely to be extremely low. There is experimental evidence that indicates the incubation period increases with a decrease in dose (McLean and Bostock 2000; Wells et al. 2007). Even extremely low levels of vaccine contamination could potentially result in a small number of infections due to the large number of animals that may be vaccinated, although they may not present clinical signs until several years after vaccination, making it difficult to trace the source of infection back to the vaccine.

The infectivity of tissues, from an animal that has died from a TSE, varies greatly and, to some extent, varies between agents (i.e. scrapie and BSE). According to the European Food Safety Authority (EFSA), milk and milk products are considered to present a negligible, or at worst, very low risk for BSE, but not for scrapie (EFSA 2008). Brain, spinal cord and other neural tissue, lymphatic tissue and certain offals are considered to be high-risk materials for the transmission of TSEs (IFST 2004).

### 3.5 Impact of disease reporting

The TSE status of a country may change once an indigenous case is reported. Due to the length of time associated with vaccine production runs, it is difficult for a manufacturer to re-establish master cell lines and master seed organisms. Locating and adapting vaccine production to alternative media or other substrates can also be difficult. It is also recognised that access to many veterinary vaccines and therapeutics is critical to maintaining the health and welfare of animals.

Although most animal products from TSE risk countries are generally considered an extremely low risk, the use of some in veterinary therapeutics, especially parenteral use in susceptible species, represents a higher risk requiring risk management. In the event that a country's TSE status is reclassified, for previously approved product, vaccine manufacturers using high risk materials derived from that country will be given a reasonable period of time to re-establish master seeds and re-source substrates from negligible risk countries or undertake other measures in compliance with this review. However, approval will be based on a case-by-case assessment taking into consideration country of origin factors, tissue of origin, date of isolation, date and origin of all cell lines, nutrient factors and/or media used, potential for cross-contamination, target species, processing, for example.

## 4 RISK FACTORS USED IN DEVELOPING REQUIREMENTS

The requirements of this review have been based on the following factors:

- country of origin (e.g. TSE status)
- species of origin (with emphasis on natural, not experimental transmission)
- tissue of origin (based on the tissue risk classification system in Appendix 1)
- target species susceptibility (including experimentally)
- method of administration (i.e. oral versus parenteral)
- manufacturing method and degree of processing
- other factors (e.g. age of the source animal, testing at slaughter and dilution of the TSE agent during either substrate or vaccine production).

### 4.1 TSE status of the country of origin

The OIE Code was amended in 2005, changing the five category system for BSE risk into a three category system. This review, previously based on the former five categories, has been updated to incorporate the current three category system.

This review will categorise countries into the following three levels of BSE risk based on Chapter 11.5 'Bovine spongiform encephalopathy' of the 2011 OIE Code (OIE 2011a).

1. negligible BSE risk country
2. controlled BSE risk country
3. undetermined BSE risk country.

The BSE status of OIE member countries is evaluated by an *ad hoc* group of experts and recommendations made to the OIE Scientific Commission for Animal Diseases. Every year a list of countries is recognised by the OIE as negligible BSE risk and controlled BSE risk.

Appendix 3 provides a copy of Resolution No. XVII adopted by the International Committee during the General Session in 2011 listing the BSE status of member countries. The current OIE list (available online at http://www.oie.int/eng/Status/BSE/en_BSE_free.htm) will be used by DAFF as the basis for determining a country's BSE risk category for the purpose of this review. All countries not listed as negligible or controlled BSE risk by the most current resolution will be considered undetermined BSE risk unless otherwise determined.

In cases where the TSE status of a country is undergoing review and has not been finalised, DAFF will base its assessment of the product on the assumption that the country's status will be downgraded. If the country is subsequently not downgraded, the application can be reassessed.

Although scrapie is an OIE notifiable disease, not all countries report their scrapie status to the OIE. Based on previous assessments, DAFF considers Australia, New Zealand and South Africa historically free from classical scrapie. Recognition of the scrapie-free status of other countries will be determined by DAFF as required. Atypical scrapie is currently considered a spontaneous TSE with possible worldwide distribution. The OIE Code specifically excludes atypical scrapie from its scrapie chapter.

CWD has been reported in Canada, the Republic of Korea and the United States. TME has been reported in Canada, Finland, Germany, the Republics of the former Soviet Union and the United States, but not since 1985 (APHIS 2002). Recognition of the status of other countries in relation to other TSEs that are not reportable to the OIE (e.g. CWD, TME) will also be determined by DAFF as required.

Those seeking to import material are strongly encouraged to critically review the country of origin of all ruminant-derived raw materials.

### 4.2 Species of origin and target species susceptibility

Animals in the family Bovidae (e.g. bovines, eland, kudu, nyala, oryx) and the family Felidae are susceptible to BSE (Kirkwood et al. 1994; Pearson et al. 1991; Pearson et al. 1992). The BSE agent causes vCJD in humans (Will et al. 1996). Other species including primates (Lasmézas et al. 2001), pigs (Meldrum 1990; Ryder et al. 2000), sheep and goats (Foster et al. 2001; Jeffrey et al. 2001) have been infected experimentally with the BSE agent. Also, one confirmed and one possible case of BSE in goats have been reported (Promed Mail 2005a; Promed Mail 2005b; Vaccari et al. 2009).

There is no evidence of natural occurrence of any form of TSE in pigs. Prior to implementing feeding controls, domestic pigs in the United Kingdom would have been potentially or actually exposed to large amounts of BSE contaminated ruminant derived meat and bone meal without incident. However, a study involving 21 pigs demonstrated experimental transmission of BSE following combined intracranial, intravenous and intraperitoneal inoculation to produce disease with an incubation period range of 69 to 150 weeks. The same study failed to produce disease in pigs retained for seven years after oral exposure to BSE affected brain at eight weeks of age (Dawson et al. 1990; Wells et al. 2003). The homogeneity of the prion protein gene has been demonstrated across 12 Asian and European and pig breeds (Lipp et al. 2004) indicating that there may not be any genetic variability in susceptibility between breeds. Further research is needed to fully evaluate susceptibility of pigs, including via other modes of administration (e.g. intra-muscular and subcutaneous) to BSE. Until then, all pig breeds will be considered equally susceptible to BSE via parenteral inoculation but not via oral exposure.

CWD, scrapie and TME have also been experimentally transmitted to cattle (Hamir et al. 2007). Dogs have not been shown to be susceptible to any TSE, naturally or experimentally. Prions from a hamster strain of scrapie, previously thought not to be infective for mice, have been shown to replicate in mice without causing clinical disease that has raised concerns of possible subclinical forms of prion infection in other species (Hill et al. 2000; Race et al. 2001).

TME affects mink and has been experimentally transmitted by intracerebral injection to sheep, cattle, monkeys and hamsters but there is no evidence of natural transmission to other species (Kretzschmar et al. 1992). CWD has been experimentally transmitted by intracerebral injection to a number of animal species including mice, ferrets, mink, squirrel monkeys, goats and cattle but has not been transmitted to cattle, sheep and goats in close contact with infected deer or to cattle or macaques via oral challenge (Belay et al. 2004; Race et al. 2009).

Species demonstrated to be naturally susceptible to TSEs (refer Appendix 2, List 1) will be considered risk species in relation to source material. Tissues from experimentally susceptible species (e.g. pigs) will not be considered risk material unless otherwise stated in this review. However, both naturally and experimentally susceptible species will be considered susceptible target species for parenteral veterinary products (refer Appendix 2, List 4).

### 4.3 Tissue of origin

The European Commission's Scientific Steering Committee opinion, *Listing of specified risk materials: a scheme for assessing relative risks to man,* as revised in January 1998, provided a categorisation of potential infectivity of different organs in BSE-infected animals. This assessment was based on scrapie titre, findings of high infectivity in the brain of BSE affected cattle, infectivity of various tissues from BSE-infected animals in mice and the presumed CJD infectivity of human dura mater and pituitary gland. Tissues were classified into four categories (high infectivity – category I, medium infectivity – category II, low infectivity – category III, and no detectable infectivity – category IV). Similarly, the 2001 revision 1 of the EMEA *Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products* also used a four-category system.

The *Note for guidance* was amended in July 2004 (EMEA/410/01 Rev. 2) to use a three-category system—high, lower and no detectable infectivity (European Medicines Agency 2004). It was also revised in 2011 (EMA/410/01 Rev. 3) to make reference to the OIE rules in relation to the BSE status of countries (European Medicines Agency 2011).

The TGA has also adopted the three-category system for its revised supplementary requirements for therapeutic goods for minimising the risk of transmitting TSEs (TGA 2004). The World Health Organization (WHO) guidelines on tissue infectivity provides a comprehensive review of the distribution of tissue infectivity and also uses the three-category system (WHO 2006).

DAFF considers the three-category system (Category A – high infectivity, Category B – lower infectivity, Category C – no detectable infectivity) to be effective and practical when applied to the extensive range of possible vaccine substrates and it has been adopted for use in this review (refer Appendix 1).

The degree of involvement of, and spread via, the lymphoreticular system with associated peripheral tissue infectivity varies between acquired TSEs such as BSE, CWD scrapie and vCJD. Peripheral tissue involvement is limited in classical BSE and is not a feature of spontaneous human

TSEs such as sporadic CJD and familial CJD. Sporadic CJD is thought to arise within the central nervous system itself, possibly as a result of a random event. Although sporadic CJD has been reported in neural tissue outside the brain (i.e. neurosensory retina, olfactory epithelium, peripheral nerve and trigeminal ganglion), it is probably the result of centrifugal spread via neural pathways.

Atypical scrapie is currently considered to be a spontaneous TSE in goats and sheep (Fediaevsky et al. 2009) and, although the tissue tropism of atypical scrapie in naturally occurring cases has not yet been fully investigated, infectivity is likely to be largely restricted to neural tissue. One study has shown low levels of infectivity in both natural and experimental ovine cases of atypical scrapie in skeletal muscle, peripheral nerves and lymphoid tissues (Andréoletti et al. 2011). Controls on the use of neural material from sheep should be sufficient to address the risk associated with atypical scrapie; however, DAFF will closely monitor developments with the disease.

Similarly, the peripheral tissue tropism of H-type and L-type BSE in experimental or natural cases has not yet been fully investigated. One study has shown low levels of L-type BSE infectivity in peripheral nerves of experimentally challenged cattle (Iwamaru et al. 2010).

### 4.4 Method of administration

Experimental inoculation via the intracerebral route has consistently been more efficient in transmission than peripheral routes. Generally, the order of decreasing transmission effectiveness is intracerebral and intraspinal routes, intravenous, intraperitoneal, subcutaneous and then ingestion. The intravenous route has been found to be relatively efficient. The oral route was considerably less efficient than any parenteral route (Phillips et al. 2000).

### 4.5 Other factors

**Age of the source animal:** As TSE infectivity accumulates in animals over a possible incubation period of several years, tissue should be sourced from young animals if practical. In the United Kingdom, the ‘over thirty month’ rule was used until 2005 as a major risk mitigation method with bovines greater than 30 months of age not permitted to be used for human or animal food.

**Method of slaughter:** Stunning by captive bolt where the cranial cavity is penetrated, can disseminate brain material into the blood stream. Non-penetrative stunning methods or electro-narcosis methods negate this risk.

**Dilution of TSE agent**: Substrates used in the vaccine production process are generally a composite of material from many animals thus diluting the TSE infectivity of any material derived from an infected animal. Multiple passages of viruses, bacteria and other organisms on TSE-free material should lead to a substantial dilution of any TSE agent originating from its initial isolation, unless replication occurs within the organism. Multiple passages of a cell line using only TSE-free substrates would only reduce infectivity derived from an infected source animal if prion replication did not occur within the cell line.

**Testing**: Although several *in vitro* diagnostic tests are available for detecting TSE agents in brain samples from infected animals, they are less sensitive than *in vivo* infectivity assays. However, *in vitro* screening of slaughtered animals may prevent the use of some animals in the later stage of incubation of disease. Should appropriate, validated *in vitro* tests for detecting TSE agents in

substrates, cell lines etc., become available, their use would be considered as a risk mitigation measure in the assessment of veterinary products.

**Manufacturing process:** The rigorous process applied to some products, such as gelatine and tallow derivatives, cannot be applied to many other products used in the manufacture of vaccines and other therapeutics. Although TSE agents are resistant to most physical and chemical treatments, the manufacturing process may provide at least some inactivation of the agent. Also, some processes such as precipitation or filtration may substantially remove protein risk material. Validation studies[ii] on removal or inactivation of the agent can be difficult to interpret, requiring consideration of the nature of the spiked material, its relevance to the natural situation, the design of the study including approximation to the commercial manufacturing process, and the method of detection (European Medicines Agency 2004). Claims of safety due to the manufacturing process, based on validation studies, will be considered but may need to be complemented by additional risk mitigation measures.

# 5 REQUIREMENTS FOR MATERIALS OF CONCERN

## 5.1 Materials derived from TSE-free countries and/or non-susceptible species

**Requirement for bovine materials derived from negligible BSE risk countries**

- There are no BSE restrictions on the use of bovine materials, other than neural tissue, from negligible BSE risk countries.

**Requirement for ovine and caprine materials derived from scrapie-free countries**

- There are no TSE restrictions on the use of ovine or caprine materials, other than neural tissue, from countries free of classical scrapie.
- There are no restrictions relevant to atypical scrapie on product other than neural material.

**Requirement for materials derived from other TSE susceptible species from countries free of the relevant TSE**

- There are no TSE restrictions on the use of materials, other than neural tissue, from species susceptible to other TSEs if derived from countries free of the relevant TSE.

**Requirement for materials derived from species not naturally susceptible to TSEs**

- There are no TSE restrictions on the use of materials from species not considered naturally or experimentally susceptible to TSEs (Appendix 2, List 3)
- There are no TSE restrictions on the use of materials, other than neural tissue, from species considered experimentally susceptible only to TSEs (Appendix 2, List 2).

Note: Refer to Section 5.3 for the requirements for neural material.

## 5.2 Products derived from multiple countries, multiple species or multiple raw materials

**Requirements on product derived from multiple countries or multiple species or multiple ingredients**

[ii] Also referred to as clearance studies.

- Where the country of origin of the product may be one of a number of countries, the requirements for that product will be that applicable to the country in the highest risk category.
- Where the product may contain material derived from more than one species, the product shall meet requirements applicable to each species for the product in question.
- Where the product is derived from more than one raw ingredient of animal origin, the product shall meet requirements applicable to each ingredient in question.

### 5.3 Neural material

DAFF considers neural material derived from TSE susceptible species to be the highest risk product for use in TSE susceptible species. All known human iatrogenic TSE transmissions to date have involved the use of brain, dura mater, cornea and pituitary gland tissues, blood or blood products, or contaminated neurosurgical equipment. Long incubation periods and difficulty in detecting the disease may mean that a country may be infected long before the disease is detected.

**Requirements for neural material**[iii]

- Bovine neural material from an undetermined BSE risk country is not permitted to be used directly or indirectly in the manufacture of products for use in any species.
- Neural material from any species naturally susceptible to TSEs (Appendix 2, List 1), regardless of country of origin, is not permitted to be used directly or indirectly in the manufacture of products for use in TSE susceptible target species (Appendix 2, List 4).
- Neural material from experimentally susceptible species (Appendix 2, List 2) from an undetermined BSE risk country is not permitted to be used directly or indirectly in the manufacture of products for use in TSE susceptible target species (Appendix 2, List 4).
- Master seed organisms, if considered an unacceptable risk due to the above use of neural material, may be re-established as detailed in Section 5.15.
- The date of creation of a master cell line may be considered, as detailed in Section 5.14, in relation to the TSE status of the country of origin of any neural material used in the creation of the cell line.

### 5.4 Milk and milk derivatives

The 2011 OIE Code does not recommend any BSE related conditions on milk and milk products, regardless of the BSE status of the cattle population. The *Note for Guidance* (EMEA/410/01 Rev. 3) states that in light of current scientific knowledge and irrespective of the geographical origin, bovine milk is unlikely to present a risk of BSE transmission. On this basis, bovine milk and bovine milk derivatives are considered to be compliant with the scope of the *Note for Guidance* provided that the milk is sourced from healthy animals and in the same condition as milk collected for human consumption (EMEA/410/01 Rev. 3).

[iii] The requirements specified for neural material also apply to other Category A tissues and their derivatives (refer Appendix 1).

According to the Phillips Report (Phillips et al. 2000), milk contains cells from the lymphoreticular system and therefore could contain TSE infective agent. However, transmission of kuru by milk was excluded. Also, no infectivity was found in milk from BSE affected cattle when tested in experimental mice by intracerebral injection, intraperitoneal injections and feeding. There is no field evidence that calves sucking BSE affected dams, or fed milk from BSE affected cows, develop BSE. Results of a maternal transmission study of BSE do not suggest the occurrence of transmission via milk (Phillips et al. 2000).

Milk, mammary glands and supra-mammary lymph glands from clinically BSE affected cows fed to mice failed to transmit the disease (Taylor et al. 1995a). Abnormal PrP could not be detected in the cell fraction of milk collected at different stages of lactation in experimentally infected cows (Everest et al. 2006) although the results for colostrum from BSE affected cows were less convincing.

It has been demonstrated that classical scrapie can be transmitted to sheep via milk and/or colostrum. Infection and pathogenesis of classical scrapie in sheep is different from BSE in cattle. Distribution of infectivity in various systems and organs of cattle is comparably more limited than that in sheep. In particular, there appears to be greater involvement of the lymphatic system in TSE pathogenesis in sheep than in cattle. Abnormal PrP has been detected in mammary glands of sheep affected with scrapie. A 'worst-case scenario' study was able to demonstrate transmission of classical scrapie using milk and colostrum from clinically affected sheep fed to lambs of the most susceptible genotype (Konold et al. 2008). Another study demonstrated that classical scrapie can be transmitted from susceptible ewes to transgenic mice via both colostrum and milk. Infectivity was associated with cellular, cream and casein-whey fractions (Lacroux et al. 2008).

The EFSA noted that both studies demonstrated that milk from asymptomatic donor ewes transmitted disease, indicating that clinically healthy, scrapie-incubating sheep may shed the TSE agent in milk (EFSA 2008). Although the EFSA did not offer any advice on the risk from milk of sheep affected with BSE or atypical scrapie, it did note that the recognised peripheral distribution of infectivity in some small ruminants infected with BSE could lead to the presence of infectivity in milk. It also noted that atypical scrapie has not been identified in peripheral tissues of infected animals and there is no epidemiological evidence of horizontal or vertical transmission.

According to a position statement by the United Kingdom's Spongiform Encephalopathy Advisory Committee, there is currently no epidemiological evidence of maternal transmission of vCJD, including via breast milk. No diagnostic test is currently available for detecting abnormal PrP in milk although research is underway to develop tests to screen for their possible presence in milk from cattle experimentally infected with BSE. Although available evidence is limited, there is a theoretical risk of transmission of vCJD via breast milk. If there is any risk, it appears to be low but cannot be excluded, warranting a watching brief (SEAC 2005).

Lactose, galactose and lactulose are highly processed milk sugars derived from whey that is derived from milk by digestion using calf rennet. Rennet is derived from calf abomasums. The rennet is considered a very low TSE risk (abomasums are Category C tissue – no detectable infectivity) and source animals are generally less than six months old. Lactose and the other sugars are extracted from the digested whey by a combination of crystallisation, separation, washing, refining, filtration and drying (in that order). The European Pharmacopoeia monograph requires that it contain no more than 70 mg/kg of protein but, in general, none is ever detected. A risk assessment conducted by the Committee for Proprietary Medicinal Products of the European Commission concluded that the BSE risk in pharmaceutical grade lactose and other similar products derived from whey was negligible (European Medicines Agency 2002). DAFF also considers milk sugars, such as lactose,

to be a negligible BSE risk. Other milk derivatives are also most likely to be a negligible or, at worst, very low BSE risk product.

Therefore, TSE restrictions will not be placed on milk sugars from any country or species. Also, TSE restrictions will not be placed on other bovine milk or milk derivatives for use in non-TSE susceptible species; nor on bovine milk and milk derivatives from negligible or controlled BSE risk countries. Sheep and goat milk and its derivatives, other than milk sugars, from classical scrapie affected countries will not be permitted for use in TSE susceptible species. All other milk and/or milk derivatives from other TSE susceptible species will require case-by-case assessment.

The requirements for colostrum will be separate to that for milk and other derivatives as there is insufficient data to determine the BSE risk associated with bovine colostrum. Ovine colostrum has been demonstrated to have significant infectivity for scrapie.

**Requirements for milk and milk derivatives, excluding colostrum**

- No TSE restrictions apply to the use of bovine milk or milk derivatives sourced from negligible or controlled BSE risk countries.
- No TSE restrictions apply to the use of pharmaceutical grade lactose, galactose, lactulose and other milk sugars.
- Milk and its derivatives, other than milk sugars, from sheep or goats in classical scrapie affected countries may not be used in the production of veterinary vaccines and therapeutics for use in naturally or experimentally TSE susceptible species (Appendix 2, List 4).
- Milk and its derivatives from other TSE naturally susceptible species (Appendix 2, List 1) from affected countries may only be used in the production of veterinary vaccines and therapeutics if they are either:
    - destined for use in species not susceptible to TSEs (Appendix 2, List 3), or
    - assessed on a case-by-case basis as acceptable after taking into consideration country and/or herd of origin factors, manufacturing process, potential for cross-contamination, target species, method of administration etc.
- Requirements for milk products, other than milk sugars, manufactured using other materials of animal origin are those also applicable to the non-milk animal materials.

**Requirements for colostrum**

- Colostrum from sheep or goats in classical scrapie affected countries and bovine colostrum from undetermined BSE risk countries may not be used in the production of veterinary vaccines and therapeutics for use in naturally or experimentally TSE susceptible species (Appendix 2, List 4).
- Bovine colostrum from controlled BSE risk countries and colostrum from other TSE naturally susceptible species (Appendix 2, List 1) from affected countries may only be used in the production of veterinary vaccines and therapeutics if they are either:
    - destined for use in species not susceptible to TSEs (Appendix 2, List 3), or
    - assessed on a case-by-case basis as acceptable after taking into consideration country and/or herd of origin factors, manufacturing process, potential for cross-contamination, target species, method of administration etc.

## 5.5 Gelatine and collagen

Gelatine and collagen are manufactured predominantly from porcine or bovine tissues such as skin/hide and bone. For bovine bone-derived gelatine, the *Note for guidance* (EMEA/410/01 Rev. 3) identifies parameters that contribute to minimising potential prion contamination (European Medicines Agency 2011). These are:

- the geographical origin of the animal derived material
- exclusion of skull and spinal cord
- exclusion of vertebrae, especially depending on the BSE status of the country of origin
- gelatine should be manufactured according to the methods outlined in the European Commission Scientific Steering Committee report (Scientific Steering Committee 2003)
- monitoring production process, batch delineation and cleaning between batches in accordance with ISO 9000
- procedures should be in place to ensure traceability and to audit suppliers of raw materials.

Due to the large number of products containing gelatine as an excipient or in a coating device, and the long shelf-life of gelatine, any recall could have serious consequences for supply of essential products.

Where possible, raw material, other than hides and skins, used in the production of gelatine and collagen should be sourced from animals not naturally susceptible to TSEs or from countries of negligible TSE risk relevant to the species of origin. DAFF recognises that the import and/or use of gelatine for both veterinary and human therapeutic products are often closely aligned. It is therefore desirable that the requirements for veterinary use be consistent with the OIE Code as well as those for human therapeutics (i.e. TGA and EMA requirements). Where there is some variation between OIE, EMA and TGA requirements, the requirements of this document will endeavor to apply the most appropriate relevant to risk mitigation and practical considerations. These requirements will also take account of other TSEs of concern.

In relation to bone derived gelatine, the EMA and TGA requirements do not permit the use of skulls or spinal cord regardless of country of origin. This is also consistent with this document's requirements on use of neural material. International standards also exclude use of vertebra from controlled BSE risk countries however EMA and OIE both restrict this exclusion to animals more than 30 months of age. The EMA restricts product from undetermined BSE risk countries to non-parenteral use only otherwise EMA requirements are the same as those for controlled BSE risk countries.

**Requirements for bone-derived gelatine and collagen of bovine origin**

***Negligible BSE risk countries***

Gelatine shall not be derived from skulls or spinal cord. No other BSE restrictions apply to bone-derived gelatine or collagen.

***Controlled BSE risk countries***

Bone-derived material shall:

- not be derived from vertebrae from animals greater than 30 months of age, skulls, spinal cord, or other specified risk materials (refer Appendix 1)
- not be cross-contaminated with other higher risk tissues
- be subjected to a process that:
    - includes all of the following steps:
        - pressure washing (degreasing)
        - acid demineralisation
        - prolonged acid or alkaline treatment
        - filtration
        - sterilisation at 138 ºC or higher for a minimum of four seconds; or
    - is outlined as satisfactory in EMEA/410/01 Rev. 3, s6.2(ii).

***Undetermined BSE risk countries***

Bone-derived material shall:

- meet the above requirements for controlled BSE risk countries; and
- either:
    - destined only for use in species not susceptible to TSEs (Appendix 2, List 3), or
    - destined only for non-parenteral use, or
    - assessed on a case-by-case basis as acceptable after taking into consideration country and/or herd of origin factors, manufacturing process, potential for cross-contamination, target species, method of administration, etc.

**Requirements for bone-derived gelatine and collagen of non-bovine origin**

There are no TSE restrictions on bone-derived gelatine and collagen derived from species not naturally susceptible to TSEs.

Requirements for material derived from bones of species naturally susceptible to TSEs (Appendix 2, List 1) (other than bovines) shall be as detailed above for bovine bone-derived material from controlled BSE risk countries.

**Requirements for hide-derived gelatine and collagen**

- Bovine hide-derived gelatine and collagen are acceptable if produced from raw material sourced from controlled or undetermined BSE risk countries, provided that appropriate assurance is given that cross-contamination with Category A and B tissues has not occurred.
- There are no TSE restrictions on other hide-derived gelatine and collagen.

### 5.6 Lanolin, derivatives of lanolin and wool derivatives

Wool and its derivatives are not considered a significant TSE risk. However, where wool is obtained from skins of slaughtered animals, other tissues such as hide, subcutaneous fat and small amounts of skeletal muscle may also be involved in the production of the derivative. Typical manufacturing processes involve either high temperature molecular distillation or prolonged exposure to very high pH that would provide a substantial reduction in TSE infectivity. The *Note for Guidance* (EMEA/410/01 Rev. 3) differentiates between shorn wool from live animals and wool derived from slaughtered animals.

**Requirements for lanolin, derivatives of lanolin and wool derivatives**

- No TSE restrictions apply to the use of lanolin, derivatives of lanolin and wool derivatives manufactured from wool of live animals.
- Processing of wool derivatives produced from slaughtered animals in scrapie affected countries should include either treatment at pH > 13 at $\geq$ 60 ºC for at least 1 hour or molecular distillation at $\geq$ 22 ºC.
- Wool derivatives that do not meet the above requirements will be assessed on a case-by-case basis taking into consideration origin, processing and potential for cross-contamination with material from high risk tissues.
- Requirements for derivatives of wool manufactured using other materials of ruminant origin (e.g. enzymes) are those requirements applicable to non-wool-derived ruminant materials.

### 5.7 Lecithin (phosphatidyl choline, phosphatidyl serine, phospholipids)

A large proportion of lecithin is derived from plant sources (Eichberg 1987). It can also be manufactured from eggs or animal-derived raw material such as spinal cord. Although lecithin is a highly processed product, the use of product derived from neural material from a TSE susceptible species in a TSE risk country could represent a significant biosecurity risk.

**Requirements for lecithin and derivatives of lecithin**

- Requirements for lecithin and derivatives of lecithin manufactured from neural material are detailed in Section 5.3 for neural material.
- The use of lecithin manufactured from all other animal sources will be assessed on a case-by-case basis, and in a manner consistent with the general requirements of this review, taking into consideration factors such as tissue, species and country of origin, target species, exposure route, processing and potential for cross contamination.

### 5.8 Highly purified proteins, peptides and amino acids

Although highly purified proteins, peptides and amino acids can be derived from non-animal protein sources, some may be sourced from animal tissues and some may be produced via a fermentation process using bovine material in fermentation media. These products are highly

processed and/or highly purified such that the likelihood of contamination is considered to be very low. Because the source and process of each product may vary considerably, a case-by-case assessment is generally required.

Purified proteins, peptides and amino acids derived from bovine bone should be subject to the same considerations as for gelatine and collagen (Section 5.5). Requirements for less refined tissue extracts including peptones are detailed in Section 5.11.

**Requirements for highly purified proteins, peptides and amino acids**

- Requirements for proteins, peptides and amino acids and other products derived from gelatine or collagen are detailed in Section 5.5 for gelatine and collagen.
- Requirements for highly purified proteins, peptides and amino acids manufactured from neural material are detailed in Section 5.3 for neural material.
- Requirements for products, represented in the form of hydrolysed proteins, peptones, and other less refined proteins and protein derivatives, derived from ruminant material, are detailed in Section 5.11 for peptones and other tissue extracts.
- The use of all other highly purified proteins, peptides and amino acids will be assessed on a case-by-case basis, and in a manner consistent with the general requirements of this review, taking into consideration factors such as tissue, species and country of origin, target species, exposure route, processing and potential for cross-contamination.

### 5.9 Tallow and tallow derivatives (e.g. glycerol, stearates)

The 2011 OIE Code recommends that no BSE restrictions be applied to the importation of tallow with insoluble protein impurities of less than 0.15 per cent in weight and derivatives made from this tallow. It also recommends no BSE restrictions on tallow from negligible BSE risk countries. For tallow that does not meet these requirements, the OIE Code recommends that source animals are subjected to an ante-and post-mortem inspection for BSE and that the product has not been prepared using BSE risk materials. Similar requirements are recommended for tallow derivatives with an additional option for those produced by hydrolysis, saponification or trans-esterification using high temperature and pressure.

Although the OIE Code considers tallow containing protein impurities of less than 0.15 per cent to be a very low risk for most end uses, even small levels of impurities in tallow derived from BSE affected animals could represent a significant risk if exposed to susceptible species via parenteral use. For tallow from controlled risk countries destined for parenteral veterinary therapeutic use in TSE susceptible target species, DAFF considers that it should not contain impurities of greater than 0.15 per cent protein in weight. In addition, it shall be derived from cattle which have passed ante-mortem and post-mortem inspections, and shall not have been prepared using specified risk materials (refer Appendix 1).

Tallow derivatives such as glycerol, fatty acids, stearates and salts manufactured by rigorous processing conditions are thought unlikely to be infectious (Taylor et al. 1995b). These processes include trans-esterification or hydrolysis at not less than 200 ºC for not less than 20 minutes under pressure and saponification with 12 M sodium hydroxide (95 ºC for not less than three hours for batch process and 140 ºC under pressure for not less than eight minutes for continuous process).

Manufacturers should note that there are alternatives to tallow-derived products (e.g. polysorbate derived from vegetable source) and many manufacturers have voluntarily changed from animal-derived to plant-derived raw materials. Wherever possible, this change should occur in line with the concluding remarks of the *Note for Guidance* (EMEA/410/01 Rev. 3), which states '…the preferred option should be to avoid the use of material derived from animals known to be susceptible (other than by experimental challenge) to TSEs in the products produced by the pharmaceutical industry'.

**Requirements for tallow**

- There are no restrictions on the use of tallow from negligible BSE risk countries.
- There are no restrictions on the use of tallow containing less than 0.15 per cent protein impurities for non-parenteral veterinary therapeutic use.
- Tallow from controlled BSE risk countries for parenteral veterinary therapeutic use in TSE susceptible target species (Appendix 2, List 4) shall:
    - contain less than 0.15 per cent protein impurities
    - originate from bovine animals that have passed ante- and post-mortem inspection
    - not have been prepared using BSE specified risk materials.
- The use of all other tallow will be assessed on a case-by-case basis taking into consideration country of origin factors, species and tissues of origin, manufacturing process, level of protein impurities, potential for cross-contamination, mode of administration and target species.

**Requirements for tallow derivatives (i.e. glycerol, stearates, fatty acids and salts)**

- There are no restrictions on the use of tallow derivatives if derived from tallow that meets the above tallow conditions.
- There are no restrictions on the use of tallow derivatives from negligible BSE risk countries.
- There are no restrictions on the use of tallow derivatives produced by hydrolysis, saponification or trans-esterification using high temperature and pressure.
- The use of other tallow derivatives will be assessed on a case-by-case basis taking into consideration country, species and tissue of origin, manufacturing process, level of protein impurities, potential for cross-contamination, mode of administration and target species.

### 5.10 Animal blood, serum and other blood products

Serum was initially considered to have no detectable infectivity. However, preliminary experimental evidence (Houston et al. 2000; Hunter et al. 2002) indicated that blood may be infectious. Foetal bovine serum (FBS) has been considered to be a potential BSE risk in human vaccines and regulatory authorities around the world have taken action to ensure that currently available vaccines are manufactured using FBS sourced from BSE negligible risk countries. Blood, previously classified in Revision 1 of the *Note for Guidance* (EMEA/410/01 Rev. 1) as having no detectable infectivity, was reclassified in Revision 2 of the *Note for Guidance* (EMEA/410/01 Rev. 2) as Category B – lower infectivity. The TGA requires that any human therapeutic that contains or has had exposure to serum or blood components during its manufacture must be fully evaluated by the TGA.

High TSE transmission rates in sheep by transfusion of both BSE (36 per cent) and scrapie (43 per cent) have been demonstrated using blood from donor sheep, with susceptible PrP genotypes, that

had been infected by oral inoculation. The donor blood was demonstrated to be infective from 20 per cent of the estimated incubation period with a gradual increase in infectivity from approximately 30 to 50 per cent of the incubation period until the clinical phase. Transmission rates did not appear to be significantly different in recipients receiving whole blood compared to buffy coat (Houston et al. 2008). Despite this, blood and blood products are still considered a relatively low TSE risk product.

Serum or serum albumins are usually used as a nutrient factor at all stages of the life of a cell line from creation to vaccine production. Serum is also used, via its use with cell lines, in the establishment of the master and working seed viruses. Blood is frequently used in the production of leptospira vaccines. It may also be used in blood agar for the culture of various bacteria and the production of some bacterial vaccines. Sheep blood is often used for blood agar.

In rodent models, TSE infectivity has been detected in both plasma and cellular components. It has also been detected in the buffy coat of experimentally infected primates and in the blood of sheep (WHO 2003). There have also been four possible cases of vCJD being transmitted via blood transfusion (Anstee 2008). Simply removing cellular material from blood may not provide a sufficient guarantee of product safety. Fractionation and leucodepletion are used in the United Kingdom as TSE risk mitigation measures for blood products (Promed Mail 2004a; Promed Mail 2004b). However, according to the WHO, leucodepletion has not been shown to reduce TSE infectivity (WHO 2003). Plasma fractionation has been demonstrated to substantially reduce TSE infectivity of plasma proteins. One instance of vCJD infection has been attributed to transfusion of a plasma product (Health Protection Agency 2009).

Control measures on blood and blood products, including serum, should be appropriate to the level of risk. There is now considerable evidence that blood from an infected sheep is likely to have a higher TSE infectivity than blood from an infected bovine. The use of bovine blood and blood derivatives, including serum, will not be permitted from any undetermined BSE risk country. Use of bovine blood and blood derivatives from controlled BSE risk countries may be permitted subject to restrictions or case-by-case assessment. The use of sheep or goat blood and blood derivatives, including serum, will not be permitted from any country affected with classical scrapie for use in TSE susceptible species.

**Requirements for blood, blood derivatives, serum, serum albumin and other serum derivatives**

- Bovine blood and blood derivatives, including serum, sourced from negligible BSE risk countries may be used without BSE restrictions.
- Bovine blood and blood derivatives sourced from controlled BSE risk countries may be used in the production of veterinary vaccines and therapeutics if either:
    - for use in species not susceptible to TSEs (Appendix 2, List 3), or
    - assessed on a case-by-case basis as acceptable after taking into consideration country of origin factors, age of source animals, status of the source herd, manufacturing process, potential for cross-contamination, availability of any TSE clearance studies[iv], target species, method of administration etc.
- The use of bovine blood, blood derivatives, serum or serum derivatives sourced from undetermined BSE risk countries in the production of veterinary vaccines and therapeutics destined for use in species susceptible to TSEs (Appendix 2, List 4) is not permitted.
- Sheep and goat blood, blood derivatives, serum or serum derivatives sourced from classical scrapie-free countries may be used without TSE restrictions.
- Sheep and goat blood, blood derivatives, serum or serum derivatives sourced from countries affected with classical scrapie may not be used in the production of veterinary vaccines and therapeutics destined for use in species susceptible to TSEs (Appendix 2, List 4).
- The use of all other blood, blood derivatives, serum or serum derivatives derived from TSE naturally susceptible species and sourced from a country affected with the disease will be assessed on a case-by-case basis after taking into consideration country of origin factors, age of source animals, manufacturing process, potential for cross-contamination, target species, method of administration etc.
- Within 12 months after the TSE status of the country of origin is downgraded, manufacturers are required to re-source or otherwise ensure the product meets the above requirements.

### 5.11 Peptones and other tissue extracts of animal origin

The most likely source of TSE contamination of bacterial vaccines would be from animal-derived material used in culture media or fermentation broths. Various animal-derived products such as tissue extracts, brain/heart infusion, casein, lactalbumin and peptones[v] are frequently used in bacterial vaccine production. These products are often of bovine origin. Casein and other milk-derived products are considered a very low risk and are frequently of New Zealand origin, further lowering the risk associated with these products (refer Section 5.4). The use of neural tissue, for example brain infusion, is considered a very high risk (refer Section 5.3).

Obtaining appropriate certification and documentation of a reasonable standard to provide confidence in country, species and tissues of peptones etc. are difficult. The quality of products ranges from product for human consumption through to rendered meat meals possibly containing material from a range of tissues and sources, possibly even downer animals and condemned

[iv] A clearance study, also referred to as a validation study, is a laboratory trial, replicating the actual commercial production process, to demonstrate its effectiveness in removing or inactivating the agent of concern.
[v] Peptones are obtained by partial hydrolysis of a protein by an acid or enzyme and are used in culture media in bacteriology. Peptones can be derived from a range of high protein animal or plant tissues.

product. Species cross-contamination at meat rendering plants may also be an issue especially if there is inadequate separation of species during rendering on a multi-species abattoir. Many tissue extracts and peptones may be derived from a mix of tissue including offal and some neural tissue.

Heat processing applied to tissue extracts will usually be inadequate to provide an appropriate level of reduction in TSE infectivity. Even the OIE endorsed treatment of 133 $^{o}$C for 20 minutes at 3 bar for meat meals will only provide a 2 to 3 log reduction in TSE infectivity (Appel et al. 2001; Safar et al. 1993; Scientific Steering Committee 1999; Taylor et al. 1994). Non-meat-meal-derived tissue extracts may undergo less severe heat treatments than rendered meat meals.

The probability of contamination of a product depends on factors such as the incidence of the TSE in the country of origin, tissues used and processing methods. In recent years, vaccine manufacturers have been more cautious in sourcing peptones, often insisting on either freedom from specified risk materials or sourcing bovine raw material from negligible BSE risk countries.

**Requirements for peptones and tissue extracts of animal origin**

- All peptones and other animal tissue–derived extracts from neural material and other Category A tissues[vi] shall comply with the requirements for neural material (refer Section 5.3).
- There are no TSE restrictions on the use of peptones and tissue extracts derived from Category C tissues in species not susceptible to TSEs (Appendix 2, List 3).
- There are no TSE restrictions on the use of peptones and other animal tissue–derived extracts from bovine tissue, other than neural material, from negligible BSE risk countries.
- Bovine tissue extracts and peptones sourced from controlled BSE risk countries[vii] may be used in the production of veterinary vaccines and therapeutics

  ONLY IF
  - no specified risk materials (Appendix 1) are used in the production of the product

  AND one of the following applies:
  - the product is destined for use in species not susceptible to TSEs (Appendix 2, List 3), or
  - no Category A or B risk materials are used in production (Appendix 1), or
  - a clearance study has been undertaken and the production process has been found to be effective at clearing TSE agent from the product, or
  - the product has been assessed as safe on a case-by-case basis after taking into consideration country of origin factors, manufacturing process, potential for cross-contamination, target species, method of administration etc.
- The use of bovine-derived peptones and tissue extracts derived from Category B tissues and sourced from undetermined BSE risk countries is not permitted.
- Peptones and tissue extracts derived from Category B tissues and sourced from other TSE naturally susceptible species (Appendix 2, List 1) in countries affected with the relevant disease are not permitted to be used in TSE susceptible target species (Appendix 2, List 4)[vii].
- The use of all other peptones and tissue extracts from TSE susceptible species will be assessed on a case-by-case basis after taking into consideration country of origin factors, tissue of origin, manufacturing process, potential for cross-contamination, target species, method of administration etc.

Note: Refer to Section 5.4 for requirements on milk-derived products.

## 5.12 Enzymes of animal origin

There have been no reports of trypsin or other enzymes being contaminated with TSE agents. However, based on inactivation data (IFST 2004; Rutala and Weber 2001), it is assumed prions would withstand the production process used for trypsin, pancreatin and other animal enzymes. Infective prion protein ($PrP^{sc}$) is also able to withstand trypsin and other enzymatic activity

[vi] The requirements specified for neural material in Section 5.3 also apply to other Category A tissues (Appendix 1).

[vii] Within 12 months of the TSE status of a source country being downgraded, manufacturers will be required to re-source the product to meet requirements unless alternative risk mitigation measures are assessed by DAFF as acceptable. This option is only available if the product had been used in veterinary vaccines and other veterinary therapeutics currently approved by DAFF. Extension to the 12 month period may be granted by DAFF on a case-by-case basis after taking into consideration anticipated time frames, commitments provided by the manufacturer, sourcing, processing, target species etc., but the total time frame should not exceed three years.

(McKinley et al. 1983; Prusiner et al. 1980). Bovine trypsin is used infrequently in vaccine production with porcine trypsin being most often used. The pancreas from which trypsin, pancreatin and several other enzymes are derived is a Category B risk tissue.

**Requirements for enzymes of animal origin**

- Enzymes of animal origin are considered tissue extracts and the requirements of Section 5.11 'Peptones and other tissue extracts of animal origin' apply.

## 5.13 Hormones of animal origin

Frequent sources of hormones include the pituitary and pineal gland (Category A tissues), placenta and adrenal gland (Category B tissues) and ovary and testis (Category C tissues). The use of pituitary-derived human growth hormone and gonadotropins has resulted in the transmission of CJD to other humans (Robinson 1996).

**Requirements for hormones of animal origin**

- Hormones of animal origin are considered tissue extracts and the requirements of Section 5.11 'Peptones and other tissue extracts of animal origin' apply.

## 5.14 Cell lines of animal origin

Until recently, only a few cell lines of neural origin have been demonstrated to be capable of $PrP^{sc}$ replication, transiently and at low levels (Dormant 1999). However, it has since been demonstrated that fibroblast cell lines, which express low levels of cellular mouse prion protein, are susceptible to infection with mouse-adapted scrapie. This study suggests that any cell line expressing normal host prion protein could have the potential to support propagation of TSE agents and that susceptibility of a cell line cannot be predicted on the basis of its level of expression of the cellular prion protein (Vorberg et al. 2004).

Expression of cellular prion protein ($PrP^{c}$) is a prerequisite for prion replication. The list of tissues capable of expressing cellular prion protein has expanded in recent years. Although cellular prion protein is found primarily in the central nervous system and in extra neural tissues such as spleen and lymph nodes (Brown et al. 2000), other tissues such as endothelial (Funke-Kaiser et al. 2001), epithelial (Pammer et al. 1999; Pammer et al. 2000), stromal and hematopoietic cells (Kaeser et al. 2001) can be added to that list.

The most likely source of contamination of the cell line is the animal from which the cell line was originally sourced (relevant only to cell lines from TSE susceptible species). As discussed previously, cell line nutrient factors such as serum are generally considered low risk products.

Information on a cell line created many years previously is often inadequate for a detailed risk assessment; however, the date and country of origin are usually available. There has been considerable debate as to when the first (index) case of BSE occurred in the United Kingdom. According to the Phillips Report, the first confirmed case was September 1985, but epidemiological analysis indicates a single point source of BSE in south-west England in the 1970's (Phillips et al. 2000). For this reason it is necessary to restrict the use of bovine cell lines of United Kingdom

origin to those created prior to 1970. All other bovine cell lines from BSE affected countries should be restricted to those created six years prior to the first reported case in the country of origin.

**Requirements for cell lines**

- All serum, enzymes and other nutrients and materials used in the establishment and/or use of the master[viii], working and production cell lines shall meet the relevant requirements of this review.
- The TSE requirements of cell lines derived from neural material or in which neural material was used to establish the cell line will be based on the TSE status of the country of origin at the time the cell line was established, and assessed in accordance with Section 5.3 for neural material.
- There are no TSE restrictions on the use of cell lines derived from Category C tissues in species not susceptible to TSEs (Appendix 2, List 3).
- There are no TSE restrictions on the use of bovine cell lines derived from tissue, other than neural material, from negligible BSE risk countries.
- No cell line derived from a Category B tissue or *in vivo* product, derived directly or indirectly from that cell line (e.g. a vaccine), will be approved for use in TSE susceptible species if the cell line is of bovine origin from an undetermined BSE risk country unless created at least six years prior to the first reported case of BSE in that country[ix].
- The use of all other cell lines from TSE naturally susceptible species (Appendix 2, List 1) in countries affected with the relevant disease will be assessed on a case-by-case basis taking into consideration country, species and tissue of origin, date of isolation, date and origin of all nutrient factors used, potential for cross-contamination and target species etc.
- In the event that the TSE status of the country of origin of the cell line is downgraded:
    - all nutrient factors etc., used with the cell line to produce vaccine after the status change shall comply with the relevant requirements of this review for each material
    - manufacturers will be required to seek a reassessment of the cell line within six months of the country's change of status.
- If deemed necessary, manufacturers will be required to change or re-establish[x] cell lines within 12 months[xi].

[viii] It is recognised that the history of substrate use for master cell lines established many years ago is often lacking yet the cell line may have been used to produce millions of vaccine doses without incident. For these cell lines, DAFF may undertake a case-by-case assessment taking into consideration the target species, the number of passages in safe substrates after exposure to the risk material, documented history of safe use in vaccines (i.e. number of doses used in countries with effective TSE surveillance programs) and any available information on the substrates (i.e. country, species, tissue and date of origin of the substrates).

[ix] The use of bovine cell lines of United Kingdom origin created after 1970 is not permitted.

[x] Re-establishment of the master cell line using safe substrates and an appropriate number of passages would only be considered, on a case-by-case basis, if it can be established that prion replication is not likely to occur in the specific cell line. Other factors such as the origin of the cell line and substrates, target species, dilution factors etc., would also need to be considered. The option for re-establishing a cell line is unlikely to be available for neural and lymphoreticular tissue based cell lines and would require substantial justification for other cell lines.

[xi] Extension to the 12 month period may be granted by DAFF on a case-by-case basis after taking into consideration anticipated time frames, commitments provided by the manufacturer, sourcing, processing, target species etc., but the total time frame should not exceed three years.

### 5.15 Master seed organisms

The risk of contamination of master seed viruses (MSV) by prions is very low, with the most probable sources being either the original animal from which the virus was isolated or the cell lines and associated nutrient factors (e.g. serum, trypsin) used to establish the master seed. The risk would be further reduced if the MSV was isolated from low risk tissues (e.g. serum). The dilution factor between the MSV and final vaccine would further minimise the risk due to a contaminated MSV provided there was no prion replication in the cell line system.

The risk of contamination of the master seed bacteria (MSB) is also very low, with the most probable sources being either the original animal from which the bacterium was isolated or the culture media used to establish the master seed. Prions would not replicate in culture media *per se.* Prions can be produced recombinantly in *E. coli* (Swietnicki et al. 1997; Swietnicki et al. 1998) and prion-like agents have been found to exist naturally in yeast (Prusiner 1998). However, replication within bacterial cells appears to be highly unlikely. The dilution factor will further minimise the risk with the final vaccine from a contaminated MSB given the absence of substrates for prion replication. It may, therefore, be possible to significantly reduce any risk by re-establishing a potentially contaminated master seed by several passages in safe media.

**Requirements for master seed virus, master seed bacteria and other master seed organisms**

- There are no TSE restrictions on the use of master seeds of bovine origin from negligible BSE risk countries or master seeds derived from other species susceptible to TSEs from countries free of the relevant disease. However, all other materials used to establish the master seed shall meet the relevant requirements of this review.
- The master seed organism will not be approved (unless re-established as detailed below) if the organism was isolated from a bovine (or the cell lines, nutrient factors or media used to establish the master seed were of bovine origin) from an undetermined BSE risk country unless created at least six years prior to the first reported case of BSE in that country[xii].
- Where the master seed organism (or the cell lines, nutrient factors or media used to establish the master seed) originated from a bovine in a controlled BSE risk country or from another TSE naturally susceptible species (Appendix 2, List 1) in a country affected with the relevant disease, it will be assessed on a case-by-case basis taking into consideration country, species and tissue from which it was isolated, date of isolation, date and origin of all cell lines, nutrient factors and/or media used, method of isolation (including dilution factors), potential for cross-contamination, target species etc.
- All media, cell lines, serum, enzymes and other nutrients and materials used in the establishment and/or use of working and production seeds shall meet the relevant requirements of this review.
- In the event that the TSE status of the country of origin of the master seed organism is downgraded:
    - all media, cell lines, nutrient factors etc., used to produce the vaccine after the status change shall comply with the relevant requirements of this review for each material
    - manufacturers will be required to seek a reassessment of the master seed organism within six months of the country's change of status
    - if deemed necessary, manufacturers will be required to re-establish the master seed within 12 months[xiii].
- Subject to a case-by-case assessment, a master seed organism considered to be an unsatisfactory risk may be re-established by an acceptable level of dilution[xiv].
  The media used shall meet all the relevant requirements of this review.

[xii] The use of bovine master seeds of United Kingdom origin created after 1970 will not be permitted.

[xiii] Extension to the 12 month period may be granted by DAFF on a case-by-case basis after taking into consideration anticipated time frames, commitments provided by the manufacturer, sourcing, processing, target species etc., but the total time frame should not exceed three years.

[xiv] In determining an acceptable level of dilution, DAFF will take into consideration the country, species and tissue from which it was isolated, date of isolation, date and origin of all cell lines, nutrient factors and/or media used, method of isolation (including dilution factors), potential for the cell lines to replicate prions, target species etc. For example, four or more passages on solid media of pure isolated colonies or six or more passages in liquid media provide a considerable dilution. As a guide, a cumulative dilution of approximately $10^{10}$ for bacterial master seed and $10^{18}$ for viral seeds would be appropriate. Passages from master to production may be considered as part of the cumulative dilution. A history of safe use in vaccines in countries with effective TSE surveillance programs may also be taken into consideration by DAFF when determining the need to re-establish a master seed and/or the appropriate level of dilution.

## 5.16 Bile and bile derivatives

Bile salts are incorporated into microbiological media and may also be used for human health foods and pharmaceutical intermediates. It is therefore possible that these products could be used indirectly in the production of veterinary products.

Bile is considered a Category C tissue (i.e. no detectable infectivity) although no bioassays have been undertaken. Bile is relatively free from adventitious contamination with fatty or proteinaceous material. Typical processing includes a combination of treatments such as boiling for 12 hours, filtration and solvent boiling steps and autoclaving in alkaline solution for several hours. Although alkaline treatment or autoclaving is not completely effective *per se* in inactivating TSE agents, the combination of alkaline hydrolysis at elevated temperatures is extremely efficient (Taylor 2003). The processing combined with the extremely low likelihood of bile being initially contaminated clearly supports the relative safety of bile derivatives. However, because there may be some variation in the effectiveness of processes used, the use of product from undetermined BSE risk countries should be assessed on a case-by-case basis. Although unprocessed (i.e. raw) and unhydrolysed powdered bile is unlikely to be used directly in the production of veterinary vaccines and other therapeutics, its TSE risk is considered to be similar to that of blood.

**Requirements for bile and bile derivatives**

- There are no TSE restrictions on the use of bovine bile derivatives from negligible or controlled BSE risk countries or bile derivatives from other species not susceptible to TSEs.
- There are no TSE restrictions on bile derivatives for non-parenteral use or on the parenteral use in non-TSE susceptible species (Appendix 2, List 3).
- The use of all other bile derivatives in producing parenteral veterinary therapeutics for use in TSE susceptible target species (Appendix 2, List 4) will be assessed on a case-by-case basis taking into consideration country, species and tissue of origin, manufacturing process, potential for cross-contamination and target species.
- Unprocessed (raw) bile shall meet the requirements of Section 5.10 for animal blood.

## 5.17 *In vitro* products with the potential for diversion to *in vivo* use

Some products imported for *in vitro* laboratory use have the potential to be used *in vivo*. This increases the risk of transmitting disease if the product is contaminated. Nonetheless, most *in vitro* products are unlikely to be diverted to *in vivo* use either due to their presentation or highly specialised purpose. The products of most concern are dehydrated culture media, peptones, blood, serum and serum products, and enzymes of animal origin.

The quarantine approved premises system, administered by DAFF, provides a level of assurance as DAFF approval must be obtained before an *in vivo* facility uses an imported animal-derived *in vitro* product on non-laboratory animals. However, despite this, import permit conditions associated with the *in vitro* product may be neglected once the product clears quarantine. It is therefore important that end users are aware that the product is for *in vitro* use only or that additional DAFF approval is required prior to *in vivo* use. This could be achieved by the use of appropriate labelling of product. Limiting product package size may also reduce the likelihood of repackaging (without the advisory labelling) by the end user.

**Requirements for *in vitro* products with the potential for diversion to *in vivo* use**

- No TSE restrictions apply to the following *in vitro* products:
  - product is not derived from a species naturally susceptible to TSEs
  - bovine-derived product from negligible BSE risk countries
  - product derived from other species naturally susceptible to TSEs but from countries free of the relevant TSE disease
  - the nature or presentation of the *in vitro* product is such that it is unlikely to be used on or exposed directly or indirectly to TSE susceptible species (Appendix 2, List 4)
  - *in vitro* products in individually packaged units of less than 20 ml or 20 g and labelled '*in vitro* use only' or equivalent wording
  - *in vitro* products whose distribution is restricted by DAFF to facilities known not to undertake any *in vivo* work[xv]
  - *in vitro* products of bovine origin derived from Category C tissues from controlled BSE risk countries
  - *in vitro* products derived from Category C tissues from other TSE susceptible species from countries with the relevant disease.
- Other *in vitro* reagents derived from Category B tissues of bovine origin from controlled BSE risk countries or from other TSE naturally susceptible species (Appendix 2, List 1) in affected countries:
  - prior to release from quarantine, the *in vitro* product shall be labelled on the smallest packaged unit as follows:
    - '*in vitro* use only' or equivalent wording, and
    - 'this product shall not be used directly or indirectly on animals without the prior approval of the Director of Quarantine. Contact the Biological Imports Program; DAFF, GPO, Box 858, Canberra, ACT 2601', and
    - shall be either:
      - supplied to a Quarantine Approved Premises required to obtain additional DAFF approval prior to any *in vivo* use of the *in vitro* product, or
      - pre-packaged in quantities of 500 ml or 500 g or less[xvi].
- All other *in-vitro* reagents will be assessed on a case-by-case basis taking into consideration the country, species and tissue of origin, processing, treatments, testing, presentation and potential for diversion to *in-vivo* use. DAFF may apply controls on end use, labelling, disposal etc, as deemed appropriate by the assessment.
- Any *in vitro* product destined for *in vivo* use shall comply with the relevant requirements of this review as it applies to the product destined for *in vivo* use.

[xv] It is expected that quarantine approved premises and other laboratories using the imported *in vitro* products will have appropriate systems of waste disposal, equivalent to the *National guidelines for waste management in the health care industry* 1999.

[xvi] Larger volumes may be considered by DAFF on a case-by-case basis dependent on the risk material involved, end use of the product and confidence that the product would not be diverted to *in vivo* use.

# APPENDIX 1 RISK CATEGORIES OF TISSUES AND FLUIDS

Adapted from: The EMEA *Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01 Rev. 3)* (EMEA 2011) and the WHO *Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies* (WHO 2006).

Note: Unless otherwise specified, the following list applies to tissues from species naturally susceptible to TSEs. Tissues not listed may be assessed on an "as needs" basis.

Note: Due to extreme tissue tropism of CWD in deer, all non-neural tissues will only be considered on a case-by-case basis.

**Category A (High infectivity)**

Brain, spinal cord, eye, retina, optic nerve, trigeminal ganglia, spinal ganglia, pituitary gland$^{\alpha}$, dura mater$^{\alpha}$, pineal gland, [skull, vertebral column]$^{\eta}$, distal ileum$^{\delta}$

**Category B (Lower infectivity)**

Ileum (proximal), lymph nodes, proximal colon, spleen$^{\iota}$, tonsil, cerebrospinal fluid, adrenal gland, distal colon, stomach, nasal mucosa, peripheral nerves, liver, lung$^{\beta}$, pancreas, thymus, esophagus, placenta, kidney(sheep)$^{\varphi}$, salivary gland, blood, blood vessels, bone marrow, mammary gland (sheep/goat), milk (sheep/goat), uterus$^{\phi}$.

**Category C (No detectable infectivity)**

Faeces, heart, mammary gland (bovine), milk (bovine)$^{\kappa}$, ovary, saliva, seminal vesicle, skeletal muscle$^{\varepsilon}$, kidney (bovine), testis, thyroid, foetal tissue$^{\phi}$, bile, bone, cartilaginous tissue, connective tissue, hair, skin, urine, semen (bovine), embryo (bovine), tongue, tendon, trachea, adipose tissue.

$\alpha$ Pituitary glands and dura mater can be considered Category A because iatrogenic CJD in humans has been associated with their use.

$\beta$ Lungs should be considered in Category A if the slaughtering method induces through the stunning or pithing method a transfer of brain material through the blood stream into the lung.

$\chi$ There is some, albeit inconclusive, evidence that circulating peripheral blood cells may transmit vCJD under experimental conditions.

$\delta$ Distal ileum is considered a specified BSE risk material in the OIE *Terrestrial Animal Health Code*.

$\varepsilon$ Accumulation of the disease-causing prion isoform in skeletal muscle was demonstrated following intramuscular inoculation of mice with scrapie infected tissue.

$\phi$ There is a higher likelihood of contamination when removing certain organs at slaughter compared to surgical or laboratory extraction. This is especially the case with the placenta and uterus therefore should be considered the same category as placenta (i.e. Category B) unless taken from non-pregnant animals.

$\eta$ Skull is entire head, excluding tongue and any other tissue specified in another category.

$\iota$ Ovine and caprine spleens may be Category A because of the finding of BSE agent in experimentally infected sheep.

$\varphi$ Classified as a lower infectivity tissue because infectivity and/or $PrP^{sc}$ have been found in human CJC (vCJD or other).

$\kappa$ Excludes colostrum. While bovine milk has been demonstrated to have no detectable infectivity, the little data there is on bovine colostrum is inconclusive.

[ ] Tissue not listed in the EMEA or WHO list.

## SPECIFIED RISK MATERIALS

BSE risk materials, referred to as specified risk materials, are listed in the 2011 OIE Code as follows:

- tonsils and distal ileum of bovine origin from a controlled or undetermined BSE risk country
- brains, eyes, spinal cord, skull and vertebral column (including mechanically separated meat from the skull and vertebral column) from cattle that were at the time of slaughter over 30 months of age originating from a controlled BSE risk country
- brains, eyes, spinal cord, skull and vertebral column (including mechanically separated meat from the skull and vertebral column) from cattle that were at the time of slaughter over 12 months of age originating from an undetermined BSE risk country
- meat and/or meat products from animals subjected to a stunning process, prior to slaughter, with a device injecting compressed air or gas into the cranial cavity or slaughtered by laceration of central nervous tissue by means of an elongated rod-shaped instrument introduced into the cranial cavity (i.e. a pithing process).

## APPENDIX 2 SPECIES SUSCEPTIBILITY LISTS

### 1. Species considered naturally susceptible to TSEs

- bovine
- ovine
- caprine[xvii]
- cervine
- feline
- mink
- primate[xviii]

### 2. Species currently considered experimentally susceptible to TSEs

- porcine[xix]

### 3. Species not currently considered naturally or experimentally susceptible to TSEs

- avian
- equine
- canine
- other species not listed in 4) below as determined by scientific review and on a case-by-case basis

### 4. Target species considered susceptible to TSEs

- bovine
- ovine
- caprine
- cervine
- feline
- mink
- primate
- porcine

[xvii] In addition to scrapie, goats are susceptible to BSE. The requirements of this policy as they relate to scrapie are considered adequate to address the relatively low risk with BSE in goats.

[xviii] Although humans are susceptible to a range of TSEs, human vaccines and other human therapeutics are not covered by this policy. Refer to the TGA for human therapeutic requirements.

[xix] Although BSE has been transmitted experimentally to pigs via intracerebral inoculation, there is insufficient evidence based on other transmission studies, that pigs are naturally susceptible to BSE.

## APPENDIX 3 BSE STATUS OF COUNTRIES

The OIE reported BSE status of member countries is based on an evaluation by an ad hoc group of experts and recommendations made to the OIE Scientific Commission for Animal Diseases. Refer to the OIE website for the most current OIE Resolution of Member Countries on the recognition of the BSE status of Members.

DAFF will make a determination of a country's BSE risk category for the purpose of this review. The most current OIE resolution (http://www.oie.int/eng/Status/BSE/en_BSE_free.htm) and any other relevant information will be used for the basis of that determination. All countries not listed as negligible or controlled BSE risk will be considered undetermined BSE risk.

## APPENDIX 4 REFERENCES


Andréoletti O, Orge L, Benestad SL, Beringue V, Litaise C, Simon S, Le Dur A, Laude H, Simmons H, Lugan S, Corbière F, Costes P, Morel N, Schelcher F, Lacroux C (2011) Atypical/Nor98 scrapie infectivity in sheep peripheral tissues. *PLoS Pathogens* 7: e1001285-doi:10.1371/journal.ppat.1001285.

Anstee DJ (2008) Lessons from the blood of sheep. *Blood* 112: 4369.

APHIS (2002) Transmissible mink encephalopathy. Animal and Plant Health Inspection Service. http://www.aphis.usda.gov/publications/animal_health/content/printable_version/fs_ahtme.pdf (Accessed 26 February 2009).

Appel TR, Wolff M, von Rheinbaben F, Heinzel M, Riesner D (2001) Heat stability of prion rods and recombinant prion protein in water, lipid and lipid-water mixtures. *Journal of General Virology* 82: 465-473.

Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi D, Matsubara T, Nakagaki T, Yamanaka H, Shirabe S, Yamada M, Mizusawa H, Kitamoto T, Klug G, McGlade A, Collins SJ, Nishida N (2011) Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nature Medicine* 17: 175-178.

Belay ED, Maddox RA, Williams ES, Miller MW, Gambetti P, Schonberger LB (2004) Chronic wasting disease and potential transmission to humans. *Emerging Infectious Diseases* 10: 977-984.

Benestad SL, Sarradin P, Thu B, Schönheit J, Tranulis MA, Bratberg B (2003) Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. *The Veterinary Record* 153: 202-208.

Béringue V, Vilotte J-L, Laude H (2008) Prion agents diversity and species barrier. *Veterinary Research* 39: 1-30.

Brown KL, Ritchie DL, McBride PA, Bruce ME (2000) Detection of PrP in extraneural tissues. *Microscopy Research and Technique* 50: 40-45.

Buschmann A, Gretzschel A, Biacabe AG, Schiebel K, Corona C, Hoffmann C, Eiden M, Baron T, Casalone C, Groschup MH (2006) Atypical BSE in Germany-proof of transmissibility and biochemical characterization. *Veterinary Microbiology* 117: 103-116.

Caramelli M, Ru G, Casalone C, Bozzetta E, Acutis PL, Calella A, Forloni G (2001) Evidence for the transmission of scrapie to sheep and goats from a vaccine against *Mycoplasma agalactiae*. *The Veterinary Record* 148: 531-536.

Collinge J (1996) New diagnostic tests for prion diseases. *The New England Journal of Medicine* 335: 963-965.

Collinge J, Whitfield J, McKintosh E, Beck J, Mead S, Thomas DJ, Alpers MP (2006) Kuru in the 21st century--an acquired human prion disease with very long incubation periods. *The Lancet* 367: 2068-2074.

Comoy EE, Casalone C, Lescoutra-Etchegaray N, Zanusso G, Freire S, Marcé D, Auvré F, Ruchoux MM, Ferrari S, Monaco S, Salés N, Caramelli M, Leboulch P, Brown P, Lasmézas CI, Deslys J-P (2008) Atypical BSE (BASE) transmitted from asymptomatic aging cattle to a primate. *PLoS ONE* 3: 1-8.

Croes EA, Jansen GH, Lemstra AW, Frijns CJ, van Gool WA, van Duijn CM (2001) The first two patients with dura mater associated Creutzfeldt-Jakob disease in the Netherlands. *Journal of Neurology* 248: 877-880.

Dawson M, Wells GA, Parker BN, Scott AC (1990) Primary parenteral transmission of bovine spongiform encephalopathy to the pig. *The Veterinary Record* 127: 338.

Dormant D (1999) Transmissible spongiform encephalopathy agents and animal sera. *Developments in Biological Standardization* 99: 25-34.

EFSA (2008) Opinion of the Scientific Panel on Biological Hazards on the human and animal exposure risk related to transmissible spongiform encephalopathies (TSEs) from milk and milk products derived from small ruminants. *The EFSA Journal* 849: 1-38.

EFSA Panel on Biological Hazards (BIOHAZ) (2011) Joint scientific opinion on any possible epidemiological or molecular association between TSEs in animals and humans. *The EFSA Journal* 9: 1945-doi:10.2903/j.efsa.2011.194.

Eichberg J. in H. F. Mark *et al*. ed., *Encyclopedia of Chemical Technology,* Vol. 14, Wiley, New York, 1987, pp 250-269

European Commission (1999) *The evaluation of tests for the diagnosis of transmissible spongiform encephalopathies in bovines*. European Commission, [Brussels].

European Commission (2008) *Report on the monitoring and testing of ruminants for the presence of transmissible spongiform encephalopathy (TSE) in the EU in 2007*. European Commission, Brussels. http://ec.europa.eu/food/food/biosafety/bse/annual_report_tse2007_en.pdf

European Medicines Agency (2002) *Lactose prepared using calf rennet: risk assessment in relationship to bovine spongiform encephalopathies (BSE).* European Medicines Agency. http://www.emea.europa.eu (Accessed 25 February 2009).

European Medicines Agency (2004) Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/02 Rev. 2 - October 2003) adopted by the Committee for Proprietary Medicinal Products (CPMP) and by the Committee for Veterinary Medicinal products (CVMP). *Official Journal of the European Union* 24: 6-19.

European Medicines Agency (2011) Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/02 Rev. 3 - July 2011) adopted by the Committee for Proprietary Medicinal Products (CPMP) and by the Committee for Veterinary Medicinal products (CVMP). *Official Journal of the European Union* 73: 1-18.

Everest SJ, Thorne LT, Hawthorn JA, Jenkins R, Hammersley C, Ramsay AM, Hawkins SA, Venables L, Flynn L, Sayers R, Kilpatrick J, Sach A, Hope J, Jackman R (2006) No abnormal prion protein detected in the milk of cattle infected with the bovine spongiform encephalopathy agent. *Journal of General Virology* 87: 2433-2441.

Fediaevsky A, Morignat E, Ducrot C, Calavas D (2009) A case-control study on the origin of atypical scrapie in sheep, France. *Emerging Infectious Diseases* 15: 710-718.

Fediaevsky A, Tongue S, Nöremark M, Calavas D, Ru G, Hopp P (2008) A descriptive study of the prevalence of atypical and classical scrapie in sheep in 20 European countries. *BMC Veterinary Research* 4: 1-24.

Foster JD, Parnham DW, Chong A, Goldmann W, Hunter N (2001) Clinical signs, histopathology and genetics of experimental transmission of BSE and natural scrapie to sheep and goats. *The Veterinary Record* 148: 165-171.

Funke-Kaiser H, Theis S, Behrouzi T, Thomas A, Scheuch K, Zollmann FS, Paterka M, Paul M, Orzechowski H-D (2001) Functional characterization of the human prion protein promoter in neuronal and endothelial cells. *Journal of Molecular Medicine* 79: 529-535.

Grassi J, Maillet S, Simon S, Morel N (2008) Progress and limits of TSE diagnostic tools. *Veterinary Research* 39: 1-12.

Hamir AN, Miller JM, Kunkle RA, Hall SM, Richt JA (2007) Susceptibility of cattle to first-passage intracerebral inoculation with chronic wasting disease agent from white-tailed deer. *Veterinary Pathology* 44: 487-493.

Health Protection Agency (2009) vCJD abnormal prion protein found in a patient with haemophilia at post mortem. Health Protection Agency. Press Release 17 Feb 2009 http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1234859690542?p=1231252394302 (Accessed 23 Aug 2011).

Hill AF, Joiner S, Linehan JM, Desbrusials M, Lantos PL, Collinge J (2000) Species-barrier-independent prion replication in apparently resistant species. *Proceedings of the National Academy of Sciences of the United States of America* 97: 10248-10253.

Hopp P, Omer MK, Heier BT (2006) A case-control study of scrapie Nor98 in Norwegian sheep flocks. *Journal of General Virology* 87: 3729-3736.

Houston F, Foster JD, Chong A, Hunter N, Bostock CJ (2000) Transmission of BSE by blood transfusion in sheep. *The Lancet* 356: 999-1000.

Houston F, McCutcheon S, Goldmann W, Chong A, Foster J, Siso S, Gonzalez L, Jeffrey M, Hunter N (2008) Prion diseases are efficiently transmitted by blood transfusion in sheep. *Blood* 112: 4739-4745.

Hunter N, Foster J, Chong A, McCutcheon S, Parnham DW, Eaton S, MacKenzie C, Houston F (2002) Transmission of prion diseases by blood transfusion. *Journal of General Virology* 83: 2897-2905.

IFST (2004) Bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD) in humans. Institute of Food Science and Technology. Information Statement Oct 2004 http://www.ifst.org/document.aspx?id=110 (Accessed 23 Aug 2011).

Iwamaru Y, Imamaru M, Matsuura Y, Masujin K, Shimizu Y, Shu Y, Kurachi M, Kasai K, Murayama Y, Fukuda S, Onoe S, Hagiwara K, Yamakawa Y, Sata T, Mohri S, Okada H, Yokoyama T (2010) Accumulation of L-type bovine prions in peripheral nerve tissues. *Emerging Infectious Diseases* 16: 1151-1154.

Jeffrey M, Ryder S, Martin S, Hawkins SAC, Terry L, Berthelin-Baker C, Bellworthy SJ (2001) Oral inoculation of sheep with the agent of bovine spongiform encephalopathy (BSE). 1. Onset and distribution of disease-specific PrP accumulation in brain and viscera. *Journal of Comparative Pathology* 124: 280-289.

Kaeser PS, Klein MA, Schwarz P, Aguzzi A (2001) Efficient lymphoreticular prion propagation requires PrP(c) in stromal and hematopoietic cells. *Journal of Virology* 75: 7097-7106.

Kim H, O'Rourke KI, Walter M, Purchase HG, Enck J, Shin TK (2001) Immunohistochemical detection of scrapie prion proteins in clinically normal sheep in Pennsylvania. *Journal of Veterinary Diagnostic Investigation* 13: 89-91.

Kirkwood JK, Cunningham AA, Austin AR, Wells GA, Sainsbury AW (1994) Spongiform encephalopathy in a greater kudu (*Tragelaphus strepsiceros*) introduced into an affected group. *The Veterinary Record* 134: 167-168.

Knight R (1998) The diagnosis of prion diseases. *Parasitology* 117: S3-S11.

Kong Q, Zheng M, Casalone C, Qing L, Huang S, Chakraborty B, Wang P, Chen F, Cali I, Corona C, Martucci F, Iulini B, Acutis P, Wang L, Liang J, Wang M, Li X, Monaco S, Zanusso G, Zou W-Q, Caramelli M, Gambetti P (2008) Evaluation of the human transmission risk of an atypical bovine spongiform encephalopathy prion strain. *The Journal of Virology* 82: 3697-3701.

Konold T, Moore SJ, Bellworthy SJ, Simmons HA (2008) Evidence of scrapie transmission via milk. *BMC Veterinary Research* 4: 1-10.

Kretzschmar HA, Neumann M, Reithmuller G, Prusiner SB (1992) Molecular cloning of a mink prion protein gene. *Journal of General Virology* 73: 2757-2761.

Lacroux C, Simon S, Benestad SL, Maillet S, Mathey J, Lugan S, Corbière F, Cassard H, Costes P, Bergonier D, Weisbecker J-L, Moldal T, Simmons H, Lantier F, Feraudet-Tarisse C, Morel N, Schelcher F, Grassi J, Andréoletti O (2008) Prions in milk from ewes incubating natural scrapie. *PLoS Pathogens* 4: 1-11.

Lasmézas CI, Fournier JG, Nouvel V, Boe H, Marce D, Lamoury F, Kopp N, Hauw JJ, Ironside J, Bruce M, Dormont D, Deslys JP (2001) Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: implications for human health. *Proceedings of the National Academy of Sciences of the United States of America* 98: 4142-4147.

Lipp O, Ritzmann M, Kixmöller M, Heinritzi K, Hensel A, Truyen U (2004) Homogeneity of the prion protein gene in various European and Asian pig breeds. *Journal of Veterinary Medicine, Series B* 51: 97-98.

McKinley MP, Bolton DC, Prusiner SB (1983) A protease-resistant protein is a structural component of the scrapie prion. *Cell* 35: 57-62.

McLean AR, Bostock CJ (2000) Scrapie infections initiated at varying doses: an analysis of 117 titration experiments. *Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences* 355: 1043-1050.

Meldrum KC (1990) Transmission of BSE to a pig. *The Veterinary Record* 127: 362.

OIE (2011) Manual of diagnostic tests and vaccines for terrestrial animals 2011. World Organisation for Animal Health (OIE). http://www.oie.int/eng/normes/mmanual/A_summry.htm (Accessed 23 August 2011).

OIE (2011a) Bovine spongiform encephalopathy. Terrestrial Animal Health Code 2011. World Organisation for Animal Health (OIE). http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.11.5.htm (Accessed 22 September 2011).

OIE (2011b) Scrapie. Terrestrial Animal Health Code 2011. World Organisation for Animal Health (OIE). http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.14.9.htm (Accessed 22 September 2011).

Pammer J, Cross HS, Frobert Y, Tschachler E, Oberhuber G (2000) The pattern of prion-related protein expression in the gastrointestinal tract. *Virchows Archiv* 436: 466-472.

Pammer J, Suchy A, Rendl M, Tschachler E (1999) Cellular prion protein expressed by bovine squamous epithelia of skin and upper gastrointestinal tract. *The Lancet* 354: 1702-1703.

Pearson GR, Gruffydd-Jones TJ, Wyatt JM, Hope J, Chong A, Scott AC, Dawson M, Wells GA (1991) Feline spongiform encephalopathy. *The Veterinary Record* 128: 532.

Pearson GR, Wyatt JM, Gruffydd-Jones TJ, Hope J, Chong A, Higgins RJ, Scott AC, Wells GA (1992) Feline spongiform encephalopathy: fibril and PrP studies. *The Veterinary Record* 131: 307-310.

Phillips N, Bridgeman J, Ferguson-Smith M (2000) Volume 2: science. The BSE Inquiry: The Report. http://collections.europarchive.org/tna/20090113222451/http://bseinquiry.gov.uk/report/volume2/toc.htm (Accessed 23 Aug 2011).

Pocchiari M (1998) Early identification of variant Creutzfeldt-Jakob disease. *British Medical Journal* 316: 563-564.

Promed Mail (2004a) CJD (new var.), blood supply - United Kingdom. ProMED Mail. http://www.promedmail.org (Archive Number 20040318.0758).

Promed Mail (2004b) CJD (vew var.), blood transmission, 2nd case - UK: suspected. ProMED Mail. http://www.promedmail.org (Archive Number 20040722.2009).

Promed Mail (2005a) BSE, goat - France 2002 (02): OIE. ProMED Mail. http://www.promedmail.org (Archive Number 20050201.0347).

Promed Mail (2005b) BSE, goat - UK 1990: suspected. ProMED Mail. http://www.promedmail.org (Archive Number 20050208.0434).

Prusiner SB (1998) Prions. *Proceedings of the National Academy of Sciences of the United States of America* 95: 13363-13383.

Prusiner SB, Groth DF, Cochran SP, McKinley MP, Masiarz FR (1980) Gel electrophoresis and glass permeation chromatography of the hamster scrapie agent after enzymatic digestion and detergent extraction. *Biochemistry* 19: 4892-4898.

Race B, Meade-White KD, Miller MW, Barbian KD, Rubenstein R, LaFauci G, Cerenakova L, Favara C, Gardner D, Long D, Parnell M, Striebel J, Priola SA, Ward A, Williams S, Race R, Chesebro B (2009) Susceptibilities of nonhuman primates to chronic wasting disease. *Emerging Infectious Diseases* 15: 1366-1376.

Race R, Raines A, Raymond GJ, Caughey B, Chesebro B (2001) Long-term subclinical carrier state precedes scrapie replication and adaptation in a resistant species: analogies to bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease in humans. *Journal of Virology* 75: 10106-10112.

Richt JA, Hall SM (2008) BSE case associated with prion protein gene mutation. *PLoS Pathogens* 4: 1-5.

Robinson MM (1996) Transmissible encephalopathies and biopharmaceutical production. *Developments in Biological Standardization* 88: 237-241.

Rutala WA, Weber DJ (2001) Creutzfeldt-Jakob disease: recommendations for disinfection and sterilization. *Clinical Infectious Diseases* 32: 1348-1356.

Ryder SJ, Hawkins SA, Dawson M, Wells GA (2000) The neuropathology of experimental bovine spongiform encephalopathy in the pig. *Journal of Comparative Pathology* 122: 131-143.

Safar J, Roller PP, Gajdusek DC, Gibbs CJ Jr (1993) Thermal stability and conformational transitions of scrapie amyloid (prion) protein correlate with infectivity. *Protein Science* 2: 2206-2216.

Schreuder BEC, van Keulen LJM, Vromans MEW, Langeveld JPM, Smits MA (1998) Tonsillar biopsy and $PrP^{Sc}$ detection in the preclinical diagnosis of scrapie. *The Veterinary Record* 142: 564-568.

Scientific Steering Committee (1999) Intra-species recycling - opinion *on :* the risk born by recycling animal by-products as feed with regard to propagating TSE in non-ruminant farmed animals. European Commission. http://ec.europa.eu/food/fs/sc/ssc/out60_en.html (Accessed 26 February 2009).

Scientific Steering Committee (2003) *Updated opinion on the safety with regard to TSE risks of gelatine derived from ruminant bones or hides*. European Commission, [Brussels]. : http://ec.europa.eu/food/fs/sc/ssc/out321_en.pdf

Scott MR, Will R, Ironside J, Nguyen HO, Tremblay P, DeArmond SJ, Prusiner SB (1999) Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. *Proceedings of the National Academy of Sciences of the United States of America* 96: 15137-15142.

SEAC (2005) *Position statement: maternal transmission of vCJD*. Spongiform Encephalopathy Advisory Committee, [London]. January 2005. http://tna.europarchive.org/20050301192925/http://www.seac.gov.uk/statements/cjdtransmissionfinal.pdf

Seuberlich T, Botteron C, Benestad SL, Brunisholz H, Wyss R, Kihm U, Schwermer H, Friess M, Nicolier A, Heim D, Zurbriggen A (2007) Atypical scrapie in a swiss goat and implications for transmissible spongiform encephalopathy surveillance. *Journal of Veterinary Diagnostic Investigation* 19: 2-8.

Simmons MM, Konold T, Simmons HA, Spencer YI, Lockey R, Spiropoulos J, Everitt S, Clifford D (2007) Experimental transmission of atypical scrapie to sheep. *BMC Veterinary Research* 3: 1-7.

Sohn HJ, Lee YH, Green RB, Spencer YI, Hawkins SAC, Stack MJ, Konold T, Wells GAH, Matthews D, Cho IS, Joo YS (2009) Bone marrow infectivity in cattle exposed to the bovine spongiform encephalopathy agent. *The Veterinary Record* 164: 272-273.

Spraker TR, VerCauteren KC, Gidlewski T, Schneider DA, Munger R, Balachandran A, O'Rourke KI (2009) Antemortem detection of $PrP^{CWD}$ in preclinical, ranch-raised Rocky Mountain elk (*Cervus elaphus nelsoni*) by biopsy of the rectal mucosa. *Journal of Veterinary Diagnostic Investigation* 21: 15-24.

Swietnicki W, Petersen R, Gambetti P, Surewicz WK (1997) pH-dependent stability and conformation of the recombinant human prion protein PrP(90-231). *Journal of Biological Chemistry* 272: 27517-27520.

Swietnicki W, Petersen RB, Gambetti P, Surewicz WK (1998) Familial mutations and the thermodynamic stability of the recombinant human prion protein. *Journal of Biological Chemistry* 273: 31048-31052.

TAFS (2007) TAFS position paper on atypical scrapie and atypical BSE. TAFS: International Forum for Transmissible Animal Diseases and Food Safety. http://www.tseandfoodsafety.org/tse.html (Accessed 23 August 2011). http://www.tafsforum.org/position_papers/TAFS_position_paper_on_atypical_scrapie_and_atypical_BSE_070516.pdf

Taylor DM (1999) Inactivation of prions by physical and chemical means. *Journal of Hospital Infection* 43: S69-S76.

Taylor DM (2003) The BSE-related safety of products manufactured by New Zealand pharmaceuticals from bovine bile for incorporation into pharmaceuticals.

Taylor DM, Ferguson CE, Bostock CJ, Dawson M (1995a) Absence of disease in mice receiving milk from cows with bovine spongiform encephalopathy. *The Veterinary Record* 136: 592.

Taylor DM, Fraser H, McConnell I, Brown DA, Brown KL, Lamza KA, Smith GR (1994) Decontamination studies with the agents of bovine spongiform encephalopathy and scrapie. *Archives of Virology* 139: 313-326.

Taylor DM, Woodgate SL, Atkinson MJ (1995b) Inactivation of the bovine spongiform encephalopathy agent by rendering procedures. *The Veterinary Record* 137: 605-610.

TGA (2004) TGA approach to minimising the risk of exposure to Transmissible Spongiform Encephalopathies (TSEs) through medicines and medical devices. Therapeutic Goods Administration. (Accessed 22 September 2011). http://www.tga.health.gov.au/docs/pdf/tsesupp.pdf

Vaccari G, Panagiotidis CH, Acin CH, Peletto S, Barillet F, Acutis P, Bossers A, Langeveld J, van Keulen L, Sklaviadis T, Badiola JJ, Andréoletti O, Groschup MH, Agrimi U, Foster J, Goldmann W (2009) State-of-the-art review of goat TSE in the European Union, with special emphasis on PRNP genetics and epidemiology. *Veterinary Research* 2009 Sep-Oct;40(5):48

Vorberg I, Raines A, Story B, Priola SA (2004) Susceptibility of common fibroblast cell lines to transmissible spongiform encephalopathy agents. *The Journal of Infectious Diseases* 189: 431-439.

Wells GA, Hawkins SA, Austin AR, Ryder SJ, Done SH, Green RB, Dexer I, Dawson M, Kimberlin RH (2003) Studies of the transmissibility of the agent of bovine spongiform encephalopathy to pigs. *Journal of General Virology* 189: 431-439.

Wells GAH, Konold T, Arnold ME, Austin AR, Hawkins SAC, Stack M, Simmons MM, Lee YH, Gavier-Widen D, Dawson M, Wilesmith JW (2007) Bovine spongiform encephalopathy: the effect of oral exposure dose on attack rate and incubation period in cattle. *Journal of General Virology* 88: 1363-1373.

WHO (2003) *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products*. ECBS 2003 World Health Organization, Switzerland. http://www.who.int/bloodproducts/publications/en/WHO_TSE_2003.pdf

WHO (2006) *WHO guidelines on tissue infectivity distribution in transmissible spongiform encephalopathies*. World Health Organization, Geneva. http://www.who.int/entity/bloodproducts/cs/TSEPUBLISHEDREPORT.pdf

Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG (1996) A new variant of Creutzfeldt-Jakob disease in the UK. *The Lancet* 347: 921-925.