# Transmissible Spongiform Encephalopathies

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## SUMMARY

The transmissible spongiform encephalopathies (TSEs) are a diverse group of fatal, neurodegenerative, diseases with long incubation periods and distinctive microscopic vacuolation in the central nervous system (CNS). The aetiological agent involves a proteinaceous infectious particle comprising a single "prion protein" (PrP) that induces normal trans-membrane PrP to undergo conformational change to an abnormal isoform that accumulates in the CNS with the neurodegenerative changes. Polymorphisms within the protein coding areas of the PrP gene in an animal species influence the propensity for normal PrP to undergo this conformational change. The change can be induced by an abnormal PrP transmitted as an infectious TSE agent, as in the six known animal TSEs, or can occur due to genetic predisposition, as in the various human genetic prion diseases and in sporadic human Creutzfeldt-Jacob disease. This unusual aetiology explains how TSEs (also called "prion diseases") may be transmitted as infectious diseases (with genetic influence of susceptibility) and also as purely genetic diseases, or occur sporadically.

Scrapie is a naturally occurring TSE of sheep and goats that has been recognised for more than 250 years, and occurs in many countries.

Bovine spongiform encephalopathy was first detected in Britain in 1985 and caused a major disease epidemic in adult cattle. Transmission was by oral exposure to a TSE agent in the ruminant-derived protein of meat and bone meal included in animal feed. A ban on the feeding of mammalian-derived protein to ruminants was implemented in Britain in 1988, and has been extended, with certain exemptions, to the remainder of the European Union as well as to Australia and New Zealand. Although there is evidence for maternal transmission at a low level, this is insufficient, to maintain BSE in the national cattle population. Several new TSEs detected in Britain since the onset of the BSE outbreak have been attributed to the BSE agent, and include exotic ungulate encephalopathy in captive exotic bovid species, feline spongiform encephalopathy in domestic and captive exotic cats, and human cases of new variant Creutzfeldt-Jacob disease that were first diagnosed in 1995.

Chronic wasting disease is a naturally-occurring TSE of deer and elk in the USA. Transmissible mink encephalopathy is a rare disease of farmed mink in the USA, Canada and Europe, associated with oral exposure to a TSE agent in feed.

### Identification of the agent

No definitive diagnostic test for any TSE agent is currently available. Confirmation of a clinical suspicion of a TSE depends on histological examination of the brain to detect distinctive vacuolation of grey matter neuropil (spongiform change) and of neuronal cell bodies. Accumulated abnormal PrP is detected in formalin-fixed CNS tissue by immunohistochemistry, or in detergent extracts of unfixed CNS tissue by immunoblotting or ELISA tests, or as disease-specific brain fibrils (so-called "scrapie associated fibrils") by transmission electronmicroscopy. Bioassay by transmission tests in ruminants or mice is impractical for routine diagnosis, due to the lengthy TSE incubation periods.

### Serological tests

No serological test is available as no specific immune response is recognised for any of the TSEs.

### Genetic tests

Epidemiological studies in various sheep breeds have identified the amino acid codons in the PrP gene where polymorphisms affect susceptibility to scrapie. PrP genotyping to select scrapie-resistant breeding stock could assist the control of clinical disease.

### Status of Australia and New Zealand

Both countries are free of the six known animal TSEs. Active and passive surveillance are conducted for scrapie and BSE, the TSEs of most importance to the livestock industries. Measures to prevent the introduction of TSEs are part of the ruminant import protocols developed by both countries.

## Introduction

The transmissible spongiform encephalopathies (TSEs) are a diverse group of fatal neurodegenerative diseases with long incubation periods and distinctive microscopic changes in the central nervous system.[1] The TSEs (also called "prion diseases") of most importance to Australian and New Zealand livestock industries are scrapie in sheep and goats, and bovine spongiform encephalopathy (BSE) in cattle. Scrapie has been recognised in sheep for more than 250 years, and occurs at a low annual incidence in many countries, but is not present in Australia or New Zealand.

BSE was first detected in British cattle in 1985,[2] and caused a disease epidemic that was both novel and economically devastating. The diagnosis since 1995 of human cases of a new variant form of Creutzfeldt-Jacob disease (nvCJD) in Britain,[3] and speculation about an aetiological link with BSE, sparked a major public health scare that seriously damaged the British beef and dairy industries. Experimental studies subsequently provided pathological and biochemical evidence that nvCJD and BSE are caused by the same agent.[4-6]

The only non-human TSEs recorded in Australia occurred as scrapie in 1952 in a small group of imported Suffolk sheep in Victoria,[7] and as feline spongiform encephalopathy (FSE) in 1991 in a cheetah imported from Britain to a zoo in Western Australia.[8] Eradication was by slaughter and quarantine, and no further cases have been diagnosed. However, spontaneous cases of spongiform encephalopathy could occur in any animal species in Australia, analogous to those of sporadic CJD (about one case per million people annually). Diagnostic methods for the TSEs include clinical examination, CNS histopathology, $PrP^{Sc}$ detection in CNS tissue, and experimental transmission.

### Aetiology

The major if not the sole component of the aetiological agent is thought to be a "*pro*teinaceous *in*fectious *(prion)* particle", which comprises a single "prion protein" (PrP).[9] Normal transmembrane "cellular PrP" ($PrP^{C}$) is induced to undergo conformational change to a very stable, protease-resistant isoform, "scrapie PrP" ($PrP^{Sc}$), that accumulates in the central nervous system (CNS) in association with the neurodegenerative changes.

### Epidemiology

There are six known TSEs of animals.

Scrapie

Scrapie occurs in sheep, goats, and moufflon, a type of primitive sheep. Natural scrapie in sheep is associated with more than 20 strains of the scrapie agent, and with polymorphisms of the PrP gene, particularly at amino acid codons 136,154 and 171.[10] Some sheep breeds such as Suffolk and Cheviot are more commonly affected than others. In Australia and New Zealand, the highly susceptible PrP genotypes have been confirmed in Merino, Poll Dorset, Suffolk and Cheviot breeds. Transmission from ewe to lamb occurs mainly during or after parturition and up until weaning, as well as prenatally. Horizontal spread also occurs, with foetal membranes a source of infection.

Bovine Spongiform Encephalopathy

The BSE epidemic in Britain resulted from oral exposure of cattle, as early as 1981, to a single stable strain of a TSE agent in the ruminant-derived protein of meat and bone meal included in animal feed. In July 1988, a ban on the feeding of mammalian-derived protein (other than milk) to ruminants was introduced in Britain. Consequently the annual incidence of confirmed BSE cases peaked at 37,489 in 1992, and then fell to about 4000 in 1997, as the epidemic subsided. By the end of 1997 a total of approximately 170,000 cases of BSE had occurred in adult cattle on some 34,300 farms in Britain. Feed contaminated with the BSE agent is the usual source of infection, and although minimal transmission from cow to calf is possible, there is no evidence of horizontal spread. There is no difference in breed susceptibility.

Coincident with the BSE outbreak, putatively related animal TSEs were diagnosed in Britain.[11]

Exotic ungulate encephalopathy was diagnosed within the family Bovidae in captive antelope (nyala, gemsbok, eland, Arabian and scimitar-horned oryx, and greater kudu),[12, 13] in Ankole cattle, and bison.

Feline spongiform encephalopathy occurred within the family Felidae in domestic cats[14, 15] and captive exotic cats (cheetah, puma, ocelot and tiger).[13]

Chronic wasting disease

Chronic wasting disease (CWD) within the family Cervidae has been recognised in mule deer and Rocky Mountain elk since 1967 and 1979, respectively, in wildlife facilities (and more recently in these species and white-tailed deer free-ranging) in Colorado and Wyoming, USA.[16,17] Like scrapie, CWD appears to spread naturally from animal-to-animal.

Transmissible mink encephalopathy

TME is a rare disease of farmed mink, that has caused 5 outbreaks on 11 ranches in the USA, beginning in 1947 in Wisconsin. TME has also been reported from Canada, Finland, East Germany and Russia.[18,19] TME transmission is linked to feed contaminated with a TSE agent of unknown origin, with possibilities including carcases of sheep or downer cows. However, attempts to experimentally infect mink orally with strains of scrapie have failed.

Human prion diseases

These include:

- sporadic CJD,
- familial CJD,
- iatrogenic CJD (from gonadotropin and growth hormone preparations derived from human pituitary glands, from corneal and dura mater grafts, and from inadequately sterilised neurosurgical instruments),
- nvCJD,
- kuru,
- Gerstmann-Sträussler-Scheinker syndrome (GSS), and
- fatal familial insomnia (FFI).

In Australia, sporadic CJD is the most common human prion disease, and iatrogenic CJD and familial clusters of CJD, FFI and GSS have been identified (Australian CJD Registry, 1998).

## Clinical Signs

Signs of the TSEs are predominantly neurological. The various human diseases usually involve prodromal personality changes, with progression to sleep disturbances, ataxia, myoclonus, dementia, emaciation, and cortical blindness. In sporadic CJD the median age of onset is 62 years, and there is rapidly progressive dementia with myoclonus, and death usually within a year.[20] In nvCJD there is a much younger age of onset, with early psychiatric disturbances and cerebellar ataxia, and a longer clinical course. Kuru, which is confined to the Fore tribe in the highlands of Papua New Guinea but has now almost disappeared, causes cerebellar ataxia and tremor, and progresses to severe motor incapacity, dementia and death, usually within a year.

The clinical signs of TSEs in animals depend on the TSE agent and the animal species affected. Neurological signs can be grouped as changes in mental status, in sensation, and in posture or movement.[21]

Scrapie

Changes in mental status (mild behavioural change, and hyperexcitability) usually mark the clinical onset of scrapie in sheep, but the predominant signs are pruritus and ataxia. Affected sheep rub against fences and other objects, and nibble compulsively at their flanks, causing skin damage and wool loss. There may also be tremor, trismus, and as the disease progresses, emaciation or occasionally obesity. Signs rarely develop in animals less than 2 years of age, and the peak incidence is at 4 – 6 years. The clinical course varies from weeks to months.

In goats the most common signs are ataxia, hyperaesthesia (increased sensitivity to sound and touch) and pruritus.[22]

Bovine spongiform encephalopathy

Apprehension, hyperaesthesia and ataxia are the main clinical signs in BSE, and at least one of these signs is present in most BSE cases; they are the most frequent changes in mental status, sensation, and posture or movement, respectively.[21] Other signs include temperament change, tremor, loss of bodyweight, and reduced milk yield. Pruritus, so highly characteristic of scrapie in sheep, is not a prominent sign in BSE. Most cases are in cattle 4 – 5 years of age, but

animals as young as 2 years may succumb. BSE usually has an insidious onset and a slowly progressive clinical course of weeks to months.

Exotic ungulate encephalopathy

Ataxia, tremor and weight loss are the most common of the various signs reported.[13]

Feline spongiform encephalopathy

As in BSE, changes in mental status (behavioural and temperament change), hyperaesthesia and ataxia are the major signs of FSE in domestic and captive exotic cats.[13,15] In domestic cats, the ataxia, particularly affecting the hind limbs, leads to a crouching gait; other signs include nodding of the head, altered grooming, tremor, trismus, hypersalivation, polydipsia and polyphagia.

Chronic wasting disease

In CWD of deer and elk, signs include emaciation, changes in mental status (behavioural change, diminished awareness, somnolence, repetitive activity, teeth grinding, and hyperexcitability), excessive salivation, and difficulty in chewing or swallowing.[16,23] Ataxia is rarely observed. A diabetes insipidus-like syndrome of polydipsia, polyuria, and low urine specific gravity in clinically dehydrated animals, occurs commonly in affected deer.

Transmissible mink encephalopathy

In TME, changes in mental status (hyperexcitability and aggressiveness initially, with somnolence and compulsive biting later) and hyperaesthesia, are followed by progressive ataxia, debilitation, and death in 2 to 7 weeks.[18]

## Pathology

The lesions and their distribution are described in detail under 'Diagnostic Tests'.

## Diagnostic Tests

Collection and processing of specimens

Any animal with progressive neurological disease should be killed in a way that avoids CNS damage. The brain, with the brainstem intact, is removed from the skull as soon as possible after death. An unfixed sample (3 – 10 g) of cervical spinal cord and/or medulla caudal to the obex, is frozen for possible detection of $PrP^{Sc}$ by Western blotting, or as scrapie-associated fibrils (SAF) by transmission electronmicroscopy.

The rest of the brain, after appropriate microbiological sampling, is fixed without distortion, in neutral buffered 10% formol saline for histological examination.

Further details on collection and processing of samples are available.[24-28]

TSE agents (prions) are *not* inactivated by ultra-violet or gamma irradiation, normal autoclaving (120$^{o}$C at 15 psi/101 kPa), aldehydes (glutaraldehyde, formaldehyde), boiling, dry heat sterilisation, ethylene oxide, acetone or alcohols.

Recommended decontamination procedures include incineration, gravity displacement or porous load (prevacuum) autoclaving (134$^{o}$C – 138$^{o}$C at 30 psi/203 kPa; holding time at temperature of 18 min for a single cycle, or 3 min for six separate cycles (some authorities advise holding times at temperature of at least 1 h), and exposure of instruments and working surfaces to sodium hydroxide (1 – 2 M), or sodium hypochlorite (2% – 3% available chlorine) for at least one hour.

Immersion of formalin-fixed tissue in 96% formic acid for one hour has been shown to reduce scrapie and CJD infectivity substantially. For biosecurity purposes, the histological reference sections of BSE and scrapie distributed to veterinary diagnostic laboratories in Australia and New Zealand, were prepared from formic acid-treated brain tissue.

Further guidelines on TSE biosecurity are available.[25, 29-32]

Histopathology

Primary diagnosis of TSEs is by histological examination of the brain. The neurohistological features of prion diseases will now be presented within a conceptual framework, which will then be applied to the specific TSEs in animals.

General

The hallmark of TSEs is the histological triad in the CNS of

- vacuolation of grey matter,
- astrocytosis, and

- neuronal degeneration, (accompanied in some cases by cerebral amyloidosis).

The distribution and severity of these changes depend broadly on the TSE agent and the species affected. Lesions are usually most prominent in the brainstem in scrapie, BSE and CWD, and in the rostral areas of the brain, including the cerebral cortex, in FSE and TME.

The subtle lesions produced in cattle experimentally infected with the scrapie agent, highlight the potential variability of TSE changes that diagnostic pathologists must always keep in mind.[19, 33, 34]

Vacuolation of grey matter

Vacuolation within CNS grey matter is the most distinctive histological feature of TSEs. It has a predilection for certain neuroanatomical nuclei, particularly in the brainstem, and is usually bilaterally symmetrical.

A numerical lesion profile for a TSE in an individual animal or a species is produced by scoring the severity of vacuolation in selected areas of the brain. Lesion profiling, originally applied to experimental scrapie in mice, to differentiate strains of the scrapie agent,[35, 36] has been adapted for study of naturally occurring TSEs in animals, including BSE, FSE and CWD.[28] The 17 neuroanatomical profile areas for BSE (see Appendix 3) represent a range of brain levels, and lesion severities scored on a scale 0 to 4.[37, 38]

In diagnostic specimens, two types of grey matter vacuolation should be sought: neuropil vacuolation (spongiform change, spongiosis), and neuronal vacuolation (perikaryonal/nerve cell body vacuolation).

(a) Neuropil vacuolation:

Neuropil vacuolation (spongiform change, spongiosis), is defined by the presence of round or oval, and occasionally confluent, small vacuoles within the grey matter neuropil. It is highly characteristic of TSEs and is due to vacuolation of neuronal processes, mainly dendrites. Neuropil vacuolation is the predominant vacuolar change in BSE, FSE, CWD, TME and many experimentally transmitted prion diseases, including scrapie and CJD. It also occurs in the cerebral cortex and thalamus of skunks and foxes with rabies.[39]

The neuropil vacuolation which is a feature of TSEs must be distinguished from "status spongiosus" within grey matter, which is a non-specific manifestation of end-stage gliosis characterised by irregular cavitation of the neuropil following extensive neuronal loss. Such change in animals is not usually due to a TSE, but is reported in CJD.[40, 41] To avoid confusion in this document, the term "status spongiosus", narrowly defined above, is not applied to other forms of CNS vacuolar change, including white matter (myelinic) vacuolation.

Grey matter neuropil vacuolation can also result from astrocytic swelling in toxic and metabolic diseases. It occurs at the junction of grey and white matter in the cerebral cortex and spinal cord in endogenous intoxications associated with hepatic and renal failure, although extensive white matter (myelinic) vacuolation of the brainstem is the predominant CNS change in these syndromes.

Artefactual grey matter vacuolation is usually perivascular or pericellular.

(b) Neuronal vacuolation:

Vacuolated neurons can be a prominent feature of TSEs, particularly in natural cases of scrapie in sheep and goats. Single or multiple vacuoles within the cytoplasm (perikaryon) of nerve cell bodies, often produce ballooned neurons with a narrow rim of cytoplasm.[2]

Vacuolated neurons also occur in normal brains of sheep, goats and cattle[42] and of deer, pigs and cats, but are usually less numerous and are certainly less widely distributed than in TSE cases. In cattle they are mainly confined to the red, oculomotor and habenular nuclei[2, 42, 43] and dorsal root ganglia (Wells, personal communication), and may occur singly in the reticular formation of the medulla and at other sites.[43] In deer they are found in the red nucleus, and in pigs and cats in the dorsal vagal nucleus. Single vacuoles are more usual than the multiple vacuoles of TSEs. Neuronal vacuolation has also been recorded in other conditions, such as progressive paresis in young Angora goats[44] and in young Rottweiler dogs.[45]

Astrocytosis

In routinely stained CNS sections, astrocytosis is most marked in natural scrapie in sheep, but is less prominent in other TSEs of animals.

Reactive astrocytes undergo hypertrophy and hyperplasia, and appear as large, vesicular, naked nuclei that can be irregularly shaped and clustered. Occasionally they form typical gemistocytes with prominent, eosinophilic cytoplasm. The extent of astrocytosis is best demonstrated by special histochemical staining (eg Cajal method) or by glial fibrillary acidic protein (GFAP) immunostaining.

Microglial proliferation, producing increased numbers of rod-shaped nuclei, can accompany astrocytosis.

Neuronal degeneration and loss

Neuronal degenerative changes other than vacuolation are not easily detected in routinely stained sections. Pyknosis is the most common of these changes and produces the shrunken, angular, deeply basophilic ("dark") neurons of simple atrophy, cell sclerosis or chronic cell disease, that are seen in scrapie.[46, 47] Shrunken ("dark") neurons are also commonly associated with post-mortem change in immersion-fixed brains, and should be identified as a pathological change only in CNS tissue that is excellently preserved, and in sections free of processing artefacts.

Neuronal loss is best demonstrated by morphometric studies.[38]

Other lesions

(a) Cerebral amyloidosis:
Amyloid plaques associated with TSEs comprise aggregates of $PrP^{Sc}$. Any aggregation of fibrillar protein assembled in β-pleated sheets can give rise to "amyloid". $PrP^{C}$ is conformationally composed of 42% α-helix and only 3% β-pleated sheet, while $PrP^{Sc}$ is 30% α-helix and 43% β-pleated sheet.
Amyloid plaques occur in 50-70% of kuru cases, and are also abundant in GSS and nvCJD, but are less frequent in other TSEs. They are found in about 5% of CJD cases, and in <5% of most TSEs in animals. In scrapie of sheep there are occasionally stellate, usually perivascular plaques of amyloid in the cerebellum, and in the midbrain and areas rostral to it.[47] Amyloid plaques have been reported in the thalamic neuropil of goats with scrapie,[48] and of cattle with BSE,[42], and in the cerebral cortex and diencephalon of many deer with CWD, but have not been reported in elk with CWD[23] or in TME.[42]

The $PrP^{Sc}$ within amyloid plaques can be demonstrated by immunohistochemistry, which also detects other CNS patterns of $PrP^{Sc}$ accumulation (including perivascular, perineuronal, axonal and synaptic).

(b) White matter changes*:*

Wallerian-type axonal degeneration, secondary to neuronal degeneration, is not a usual feature of TSEs, but is reported in FSE and in elk with CWD.

Focal spongiosis of white matter (myelinic vacuolation) is a non-specific change seen as elongated, irregular spaces within white matter tracts, and occurs in some animals with TSE. The form and white matter location of these vacuoles distinguish them from the smaller, round, grey matter neuropil vacuoles characteristic of TSEs.

Focal spongiosis of white matter within the substantia nigra in the midbrain, and sometimes extending rostrally to the thalamic radiation and the internal capsule, occurred in 13.5% of cattle with BSE, and in 18% – 29% of BSE-negative cattle with signs of neurological disease in Britain; its diagnostic significance is unclear.[28, 43, 49, 50]

Similar vacuolation was seen in the white matter radiation from the dorsolateral thalamus in sheep with neurological signs.[51] It was seen in 17 of 226 sheep and 22 of 383 goats, all mature and scrapie-free, in a systematic survey of animals in Australian quarantine stations; most of these animals with focal spongiosis did not have neurological signs.[52]

Extensive myelinic vacuolation ("myelin oedema") within the CNS can be due to idiopathic, toxic or metabolic diseases, including endogenous intoxications associated with hepatic or renal failure. Vacuolation of white matter is also a feature of myelinolytic diseases such as spinal myelinopathy in Murray Grey cattle, and multifocal necrotising encephalopathy in Limousin and Simmental cattle.

Severe artefactual vacuolation of white matter can occur in brains of cattle, rats, dogs and monkeys, (but not of pigs) held in 50-90% (but not when held in 100%) ethanol for more than 12 hours before processing.[53, 54]

## Differential diagnosis

Neuropathological findings in British cattle, clinically suspected but not confirmed as having BSE, included no significant lesions (39-62%), focal spongiosis of white matter (18-29%), inflammatory disorders, mainly listeriosis (8-30%), and other lesions (3-21%) including tumours (0-3%), polioencephalomalacia (1-2%), and idiopathic brainstem neuronal chromatolysis (0-7%).[28,43, 49, 55, 56]

In other countries, including Australia and New Zealand, similar neuropathological surveys of endemic CNS disease in sheep and adult cattle provide evidence of freedom from scrapie and BSE[57, 58] .

### Scrapie

Neuronal vacuolation and astrocytosis are more prominent than neuropil vacuolation in natural cases of scrapie in sheep and goats, and each of these changes can vary in distribution and severity. This variable lesion profile may be due to different scrapie strains, and host factors including breed and PrP genotype.

Neuronal vacuolation is most marked in the medulla (especially in the dorsal vagal nucleus at the obex), pons and midbrain.

Neuropil vacuolation (spongiform change) within grey matter is probably the most variable of the CNS changes in natural scrapie,[46] and may be obscured by autolysis, and tissue disruption during processing.[59] Consequently, it has been attributed less diagnostic importance in scrapie than *neuronal vacuolation*, which is well preserved, often in the presence of severe tissue artefacts. Nonetheless, neuropil vacuolation should always be sought.

Seven recently described patterns of neuropil vacuolation in sheep with scrapie, comprise various degrees of rostral extension of this change from the obex to the cerebral cortex.[47] Rostral lesions (in the cerebral cortex, corpus striatum and septal area) are not confined to those cases with the most severe vacuolation in the brainstem.

Astrocytosis is usually severe where neuronal and neuropil vacuolation are severe. However, astrocytosis can be prominent within the granule cell layer of the cerebellum in the absence of vacuolation, and there can be gemistocytosis associated with granule cell loss.[47, 48]

Neuronal degeneration, producing pyknotic ("dark") neurons, particularly in areas rostral to the midbrain, is a consistent feature of scrapie in sheep[47] and goats.[48]

### Bovine spongiform encephalopathy

Neuropil vacuolation predominates over neuronal vacuolation, and is the most striking and diagnostically significant change in BSE.[38] The topographical distribution and relative severity of both types of vacuolation are remarkably constant (see Appendices 2 and 3), and are consistent with infection by a single strain of BSE agent.

Neuropil vacuolation is most marked in the spinal cord (dorsal horn), medulla (solitary tract nucleus, spinal tract nucleus of the trigeminal nerve, dorsal vagal nucleus, reticular formation, and olivary nucleus), pons, midbrain (central/periventricular grey matter), thalamus and hypothalamus.[28, 38] Changes decrease in severity caudo-rostrally from the midbrain.

Examination of the solitary tract nucleus and the spinal tract nucleus of the trigeminal nerve in a single transverse section of the medulla at the obex, detects neuroparenchymal vacuolation (comprising mainly neuropil vacuolation) in 99.6% of BSE cases.[59]

Neuronal vacuolation is most prominent in the vestibular nuclear complex and red nucleus in BSE.[42]

Astrocytosis (hypertrophic astrocytes and occasional gemistocytes) is rarely as severe as in natural scrapie. Neuronal degeneration is seen infrequently. Neuronal loss was demonstrated in one morphometric study, in which the number of neurons in the vestibular nuclear complex of cattle with BSE was about half that of control animals.[60]

### Exotic Ungulate Encephalopathy

There are differences in the relative severity of involvement of certain neuroanatomical nuclei compared to BSE,[11] for example in the nyala case, neuropil and neuronal vacuolation in the dorsal vagal nucleus were more intense than occurs in BSE.[61]

### Feline spongiform encephalopathy

Neuropil vacuolation usually predominates over neuronal vacuolation in FSE. It is most

prominent rostrally, and involves the dorsolateral cerebral cortex (deep layers), corpus striatum, thalamus, medial geniculate nucleus, and cerebellar cortex.[15]

Neuronal vacuolation has a caudal predilection, for the dorsal vagal nucleus, raphe nucleus, vestibular nuclear complex, red nucleus, and the occasional neuron in the spinal cord.[15] In a case of FSE in a puma, neuronal vacuolation was the most striking change.[62]

Astrocytosis and an increase in numbers of rod-shaped microglia also occur in FSE.[15]

In domestic cats with FSE, there was vacuolation of white matter mainly in the medulla, associated with axonal degeneration, particularly in the pyramidal tracts.[15] A puma with FSE had some axonal degeneration in spinal cord white matter,[62] and a cheetah had widespread axonal degeneration and demyelination of all tracts in the spinal cord.[8]

Chronic wasting disease

Neuropil vacuolation is the most striking change in deer and elk with CWD and is usually accompanied by neuronal vacuolation.[23] These changes are most prominent in the dorsal vagal nucleus, hypothalamus, and olfactory bulb and tubercle. Examination of these areas is sufficient to establish a diagnosis of CWD, although lesions are more severe and consistent in deer than in elk. The prominence of changes in the medulla (dorsal vagal nucleus and solitary tract nucleus), pons, midbrain (central grey matter) thalamus and hypothalamus in CWD, is similar to that in scrapie and BSE. However, the severe involvement of the olfactory bulb and tubercle in deer and elk with CWD, has not been described in cattle with BSE, and is uncommon in sheep or goats with scrapie.

Severe neuronal vacuolation occurs in the supraoptic and paraventricular nuclei in deer and elk, and may be associated with the diabetes insipidus-like syndrome in CWD. However, the supraoptic nucleus is a common site of artefactual neuronal vacuolation in many animal species.

Other changes include astrocytosis associated with neuropil vacuolation particularly in the thalamus and brainstem, neuronal degeneration ("dark" neurons) in the cerebral cortex, and neuronal loss in the thalamus.[23]

In elk with CWD, there can be mild axonal degeneration in the cerebrum and cerebellum.

Transmissible mink encephalopathy

Neuropil vacuolation, neuronal degeneration ("dark" neurons) and astrocytosis are the essential changes in TME. They are most severe rostrally, and involve the cerebral cortex (middle and deep layers), corpus striatum, thalamus, hypothalamus and medial geniculate nucleus. Lesions decrease in severity rostro-caudally from the midbrain (central grey matter and caudal colliculus) to the medulla (vestibular nuclear complex, dorsal vagal nucleus, hypoglossal nucleus, and lateral reticular nucleus), and spare the cerebellum and spinal cord.[18]

Neuronal vacuolation is much less common than neuropil vacuolation, and is found mainly in the brainstem.

## Detection of prion protein (PrP)

Tests for PrP in CNS samples are carried out at the Australian Animal Health Laboratory. In the future, PrP detection in specimens other than CNS tissue, for example lymphoid tissue, including tonsillar or third eyelid biopsies, may allow TSE diagnosis in the live animal (currently available only by brain biopsy) and preclinical diagnosis.

$PrP^{C}$ is soluble in detergent and is susceptible to protease digestion, whereas $PrP^{Sc}$ sediments in detergent and is partially resistant to protease digestion. Procedures to detect $PrP^{Sc}$ in unfixed (fresh or frozen) tissue utilise these properties.

Immunohistochemistry

Immunostaining of usually formalin-fixed, but also unfixed cryostat preparations of CNS tissue identifies the topographical and cellular localisation of accumulated $PrP^{Sc}$. Optimal results require pretreatment steps to unmask PrP epitopes in formalin-fixed tissue sections, including hydrated autoclaving, and proteinase K or formic acid treatment. Sections are then stained in an indirect immunoperoxidase test using monoclonal antibody to a PrP epitope. This technique can be applied to mildly autolysed tissue that is unsuitable for routine histological evaluation.

### Scrapie associated fibrils (SAF)

SAF are disease-specific, ultrastructural markers for TSEs that comprise $PrP^{Sc}$. They are demonstrated in detergent and proteinase K-treated extracts of unfixed CNS tissue by negative stain transmission electronmicroscopy.[63, 64] SAF can be detected in autolysed tissue.

### Western blotting

Immunoblotting is the most specific technique for TSE diagnosis. After detergent extraction of $PrP^{Sc}$ from unfixed CNS tissue, proteinase K digestion converts 33-35 kDa $PrP^{Sc}$ to 27-30 kDa forms, which are identified both by the altered molecular weight of the gel bands produced, and by the immunostaining of these bands using anti-PrP antibody (available antisera do not distinguish between $PrP^{C}$ and $PrP^{Sc}$).[65] Three gel bands correspond to unglycosylated, monoglycosylated or diglycosylated $PrP^{Sc}$. Immunoblotting has the potential to distinguish scrapie strains on the basis of these glycosylation patterns,[66] but this is not yet a routine operational method. Immunoblotting can be applied to autolysed tissue, as degradation of $PrP^{Sc}$ is limited by its partial resistance to proteolysis.[67] For BSE a Western blot procedure has been developed and extensively validated with high sensitivity and specificity.[68,69] This method is currently used for surveillance in Switzerland and it has been shown to detect even pre-clinical cases of BSE.[69]

### Other

Enzyme-linked immunosorbent assay (ELISA) and dot blotting are other methods of detecting PrP. For BSE three ELISA procedures have been developed and extensively validated, of which two showed high sensitivity and specificity[68].

### Other diagnostic methods

Identification of 14-3-3 protein
Identification of 14-3-3 brain protein in cerebrospinal fluid,[70, 71] and detection of metabolites in urine, are new techniques currently being assessed for use in clinical diagnosis.

Bioassay
Detection of a TSE agent by transmission tests in sheep, goats, cattle or mice, is a lengthy procedure. It has been part of the Australian and New Zealand quarantine requirements for importation of sheep and goat embryos from scrapie-affected countries.

### Australian and New Zealand diagnostic standard for TSE exclusion (see Appendix 1)

This standard for TSE exclusion involves histological examination of transverse sections of the brainstem at three standard sites: the obex, caudal cerebellar peduncles, and midbrain (See Appendices 1, 2 and 3). At present the standard in New Zealand is to look at just one site, the section through the obex. If the histological evaluation is not clearly "TSE Negative" and there is no alternative diagnosis for the neurological signs, CNS specimens are sent to the Australian Animal Health Laboratory for PrP testing.

Evaluation of these three standard sites can be part of the routine histological examination of any animal brain, but is mandatory for TSE exclusion in cases of progressive neurological disease. The Australian and New Zealand National TSE Surveillance Programs target sheep and cattle over 2 years of age.

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# APPENDICES

## Appendix 1

### Australian and New Zealand diagnostic standard for TSE exclusion

Exclusion of transmissible spongiform encephalopathy (TSE) should be part of the examination of the brain from any animal with a progressive neurological disease.

### Specimens

The brain, with the brainstem intact, is removed from the skull as soon as possible after death.

An unfixed sample (3-10 gm) of cervical spinal cord and/or medulla caudal to the obex, is frozen for possible testing for prion protein (PrP).

The rest of the brain, after appropriate microbiological sampling, is fixed without distortion, in neutral buffered 10% formol saline for histological examination.

### Standard brain sites for histological examination

Transverse sections of the brainstem are made at the following three standard sites for TSE exclusion. Evaluation of these three sites can be part of the routine histological examination of any animal brain, with examination extended to other brain sites, as required.

- Standard Site 1: Medulla at the obex.
- Standard Site 2: Medulla through the caudal cerebellar peduncles.
- Standard Site 3: Midbrain through the rostral colliculi.

### Histological changes in TSE

The three standard brain sites are evaluated for the presence of the following histological changes suggestive of TSE:

- Vacuolation of grey matter neuropil (spongiform change), and/or vacuolation of neurons (vacuolation of neuronal perikarya/nerve cell bodies).
- Astrocytosis.
- Neuronal degeneration ("dark" neurons) and / or neuronal loss.
- Amyloidosis.

### Histological diagnosis and reporting

TSE Positive

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

Scrapie:

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

Bovine Spongiform Encephalopathy:

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

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

Diffuse vacuolation of white matter (myelinic vacuolation) is not a feature of natural scrapie or BSE.

Distinction must be made between true spongiform change within grey matter, and vacuolation that is an artefact of fixation or processing.

TSE Pending

- Equivocal vacuolation of grey matter neuropil and/or neurons.

TSE Unsuitable specimen

- Severe autolytic change.
- Inadequate representation of the standard sites for TSE exclusion, or of the neuroanatomical profile areas at these sites.

TSE Negative

- No vacuolation of grey matter neuropil or neurons at the three standard sites.

Further testing required

If the histological TSE evaluation is not clearly "TSE Negative" and there is no alternative diagnosis for the neurological signs, CNS specimens are sent to the Australian Animal Health Laboratory for PrP testing.

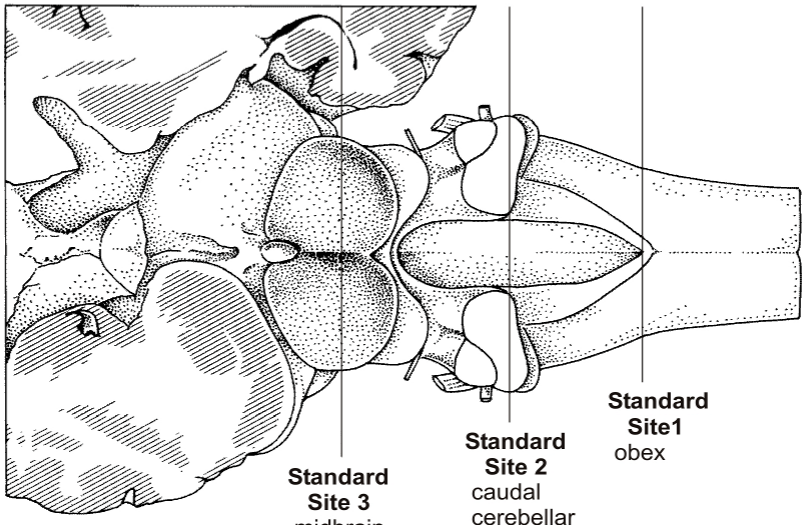
Accumulated abnormal PrP are detected in formalin-fixed CNS tissue by immunohistochemistry, or in detergent extracts of the unfixed CNS sample by immunoblotting or ELISA, or as disease-specific brain fibrils ("scrapie associated fibrils") by transmission electronmicroscopy. Bioassay by transmission tests in ruminants or mice is available but is a lengthy procedure.

Report of TSE Exclusion

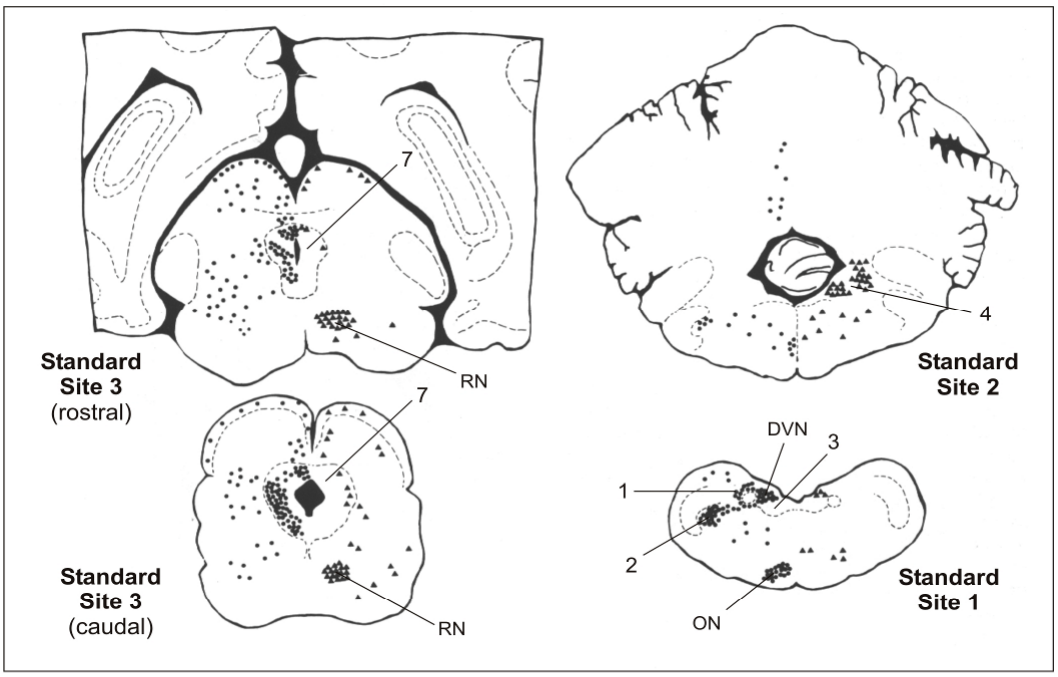
In all cases of progressive neurological disease in animals where TSE lesions have been excluded by histological examination of the standard brain sites, the laboratory report should include the following statement of TSE exclusion:

"TSE Negative - No histological lesions suggestive of transmissible spongiform encephalopathy (TSE) detected at the brain sites specified in the Australia and New Zealand Standard Diagnostic Protocols, Transmissible Spongiform Encepholopathies."

# Appendix 2. **Standard brain sites for TSE exclusion**




**Dorsal view of bovine brainstem and the three standard sites.**



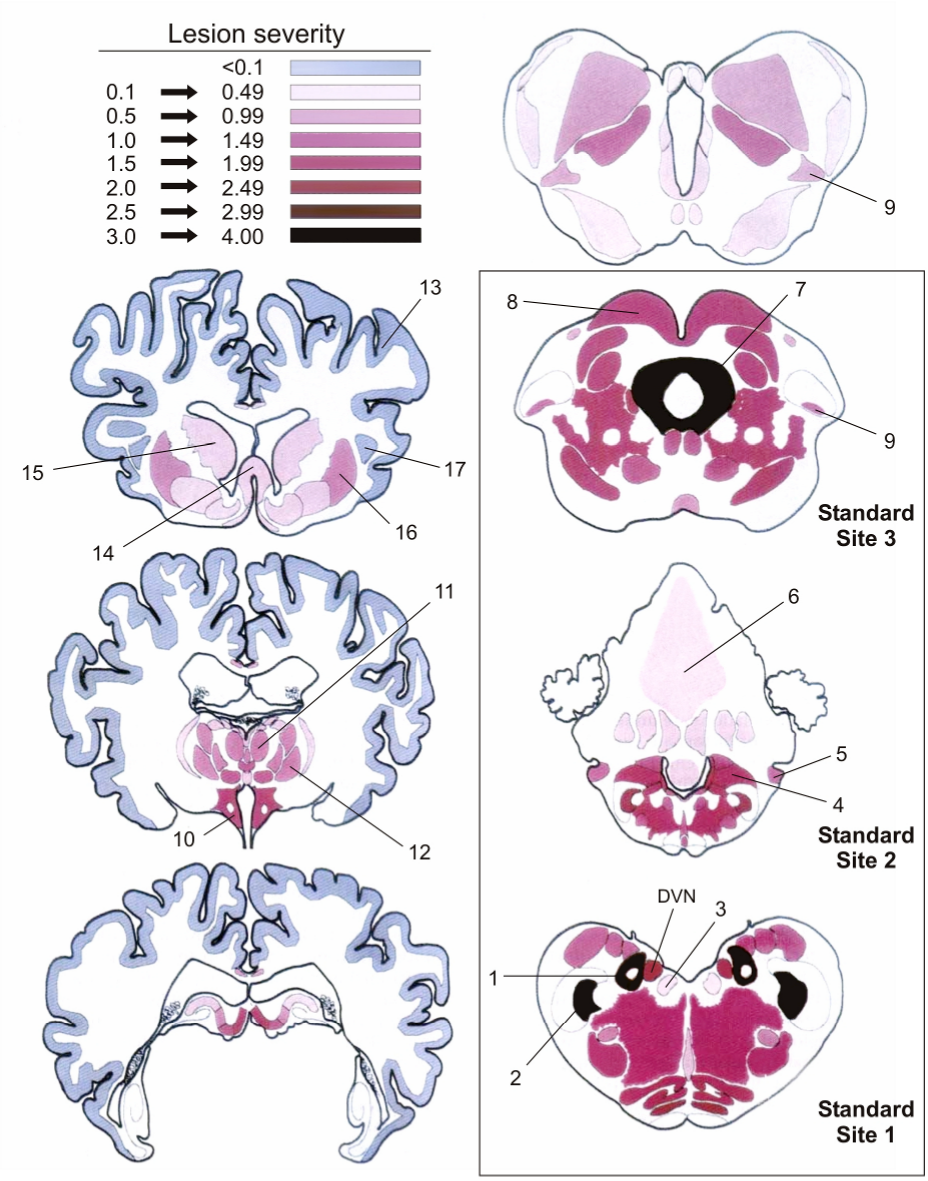

**Transverse sections of bovine brainstem at the three standard sites.**

Vacuolation in bovine spongiform encephalopathy (BSE):
- • (left half) = Grey matter neuropil vacuolation (spongiform change).
- ▲ (right half) = Neuronal vacuolation.

*After Wells GAH et al (1991) Brain Pathology 1: 69-78.*

| Code | Area |
|---|---|
| 1 | Nucleus of solitary tract |
| 2 | Nucleus of spinal tract of trigeminal |
| 3 | Hypoglossal nucleus |
| 4 | Vestibular nuclear complex |
| 7 | Central grey matter |
| DVN | Dorsal vagal nucleus |
| ON | Olivary nucleus |
| RN | Red nucleus |

# Appendix 3. **Lesion profile: Bovine Spongiform Encephalopathy**




**Distribution and relative severity of grey matter neuropil vaculation (spongiform change) and/or neuronal vaculation in BSE.**

Brain sites shown include the three standard sites* for TSE exclusion (*figures are not to scale)*:
- frontal, parietal and occipital cerebrum (*left of page*).
- diencephalon, mesencephalon*, medulla/cerebellum* and medulla (obex)* (*right of page*).

The 17 BSE profile areas represent a range of brain levels and lesion severities (scores 0-4).

*After Wells GAH and Wilesmith JW (1995) Brain Pathology 5: 91-103.*

| Code | BSE profile area | Score |
|---|---|---|
| 1 | Nucleus of solitary tract | 3.0 |
| 2 | Nucleus of spinal tract of trigeminal | 3.0 |
| 3 | Hypoglossal nucleus | 0.4 |
| 4 | Vestibular nuclear complex | 1.6 |
| 5 | Cochlear nucleus | 0.5 |
| 6 | Cerebellar vermis | 0.2 |
| 7 | Central grey matter | 3.0 |
| 8 | Rostral colliculus | 1.6 |
| 9 | Medial geniculate nucleus | 0.6 |
| 10 | Hypothalamus | 1.7 |
| 11 | Nucleus dorsomedialis thalami | 0.6 |
| 12 | Nucleus ventralis lateralis thalami | 0.7 |
| 13 | Frontal cortex | < 0.1 |
| 14 | Septal nuclei | 0.2 |
| 15 | Caudate nucleus | 0.2 |
| 16 | Putamen | 0.6 |
| 17 | Claustrum | < 0.1 |