Transmissible Spongiform Encephalopathies

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Part 1. Diagnostic Overview

Summary

The transmissible spongiform encephalopathies (TSEs) are fatal, neurodegenerative disorders affecting a variety of mammalian species. They are characterised by long incubation periods and distinctive vacuolation within the central nervous system (CNS). TSEs (also known as 'prion diseases') are defined by the accumulation of a conformationally-altered, host-membrane glycoprotein called 'prion protein' (PrP), notably in the CNS and lymphoid tissues. Evidence suggests that this abnormal PrP isoform is the principal or sole component of 'prions,' the proteinaceous infectious particles that transmit these diseases. Polymorphisms and mutations within the PrP gene can influence the propensity for normal PrP to undergo conformational change. The TSEs can be transmitted as infectious diseases, as in the six known animal TSEs, but also occur spontaneously, as in human sporadic Creutzfeldt-Jacob disease (CJD), and as purely genetic disorders, as in the various human genetic prion diseases.

Scrapie is a naturally-occurring TSE of sheep and goats that has been recognised for more than 250 years, and occurs in many countries, but not in Australia or New Zealand.

Bovine spongiform encephalopathy (BSE) 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. There is no evidence of horizontal transmission and little data supporting the existence of maternal transmission. Several new TSEs detected in Britain since the onset of the BSE outbreak, and attributed to the BSE agent, include exotic ungulate encephalopathy in captive exotic bovid species, feline spongiform encephalopathy (FSE) in domestic and captive exotic cats, and human cases of a new form of CJD termed variant CJD (vCJD), which was first diagnosed in 1995.

Chronic wasting disease (CWD) is a naturally-occurring TSE of cervids (deer, elk and moose) in the USA and Canada. Transmissible mink encephalopathy (TME), a rare disease of farmed mink in the USA, Canada and Europe, is associated with oral exposure to a TSE agent in feed, with the last outbreak reported in 1985.

The only non-human TSEs recorded in Australia occurred as scrapie in 1952 in a small group of imported Suffolk sheep in Victoria, as FSE in 1991 in a cheetah imported from Britain to a zoo in Western Australia, as FSE in 2002 in an Asiatic golden cat imported

from Germany to Melbourne Zoo, and as atypical/Nor98 scrapie in a sheep during 2010. In New Zealand, there have been two incursions of scrapie in imported sheep (in 1952-54 and 1976-77). A case of atypical/Nor98 scrapie was detected in a sheep in New Zealand in 2009.

Exclusion of TSE should be part of the examination of the brain from any species of animal with a progressive neurological disease.

Clinical diagnosis of a TSE may be confirmed by histological detection of distinctive vacuolation of CNS grey matter neuropil (spongiform change) and neuronal cell bodies and/or by detection of disease-specific accumulations of abnormal PrP; in formalin-fixed CNS tissue by immunohistochemical (IHC) methods; in unfixed CNS tissue by immunoblot (also known as Western blot) or enzyme-linked immunosorbent assay (ELISA) tests, or in lymphoid tissue from sheep, goats or cervids by either immunoblot or ELISA tests; in CNS tissue as so-called 'scrapie-associated fibrils' (SAF) by transmission electron microscopy; or in various tissues or secretions such as milk or urine by serial protein misfolding cyclic amplification (sPMCA), currently an experimental procedure. Bioassay by transmission tests in ruminants or mice is impractical for routine diagnosis, due to the lengthy incubation periods of TSEs. No serological test is available as no specific immune response is recognised for any of the TSEs.

Aetiology

The transmissible spongiform encephalopathies (TSEs) are fatal, neurodegenerative disorders affecting a variety of mammalian species. They are characterised by long incubation periods and distinctive vacuolation within the central nervous system (CNS).[5] TSEs (also known as 'prion diseases') are defined by the accumulation of a conformationally-altered, host-membrane glycoprotein called 'prion protein' (PrP), notably in the CNS and lymphoid tissues.

Normal, 'cellular PrP' (PrP^{C}) is induced to undergo conformational change to a very stable, protease-resistant isoform, 'scrapie PrP' (PrP^{Sc}). Evidence suggests that PrP^{Sc} is the principal or sole component of prions, the *pro*teinaceous *in*fectious particles that transmit these diseases.[5,6]

There are six known TSEs of animals: scrapie of sheep and goats, bovine spongiform encephalopathy (BSE), exotic ungulate encephalopathy, feline spongiform encephalopathy (FSE), chronic wasting disease (CWD) of cervids (deer, elk and moose), and transmissible mink encephalopathy (TME).

The TSEs of most importance to Australian and New Zealand livestock industries are classical scrapie in sheep and goats, BSE in cattle and CWD of cervids.

Human prion diseases comprise sporadic Creutzfeldt-Jacob disease (CJD); inherited forms, including familial CJD, Gerstmann-Sträussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI); and acquired forms, including iatrogenic CJD (transmitted via gonadotrophin and growth hormone preparations derived from human pituitary glands, via corneal and dura mater grafts, and via inadequately sterilised neurosurgical instruments), kuru, and the new variant form of CJD (vCJD).[5]

BSE, exotic ungulate encephalopathy, FSE and human vCJD are caused by the same prion agent.

Clinical Signs

The clinical signs of TSEs in animals depend on the TSE agent and the animal species affected. Neurological signs can be classified as changes in mental status, in sensation, and in posture or movement.[7]

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 obesity. The clinical course varies from weeks to months.

In goats, the most common signs are ataxia, hyperaesthesia and pruritus.[8]

Atypical scrapie

Atypical/Nor98 scrapie has been detected during surveillance testing of apparently-healthy sheep and goats at slaughter, and by testing fallen stock.[9] In sheep, changes in mental status (behavioural change), ataxia and weight loss are reported, but not the pruritus and wool loss seen in classical scrapie.[10-13]

Bovine spongiform encephalopathy

Apprehension, hyperaesthesia and ataxia are the main clinical signs in BSE, and at least one of these is present in most BSE cases; they represent the most frequent changes in mental status, sensation, and posture or movement, respectively.[7] Other common neurological signs include temperament change and tremor. There may also be loss of bodyweight, and reduced milk yield. Pruritus, so highly characteristic of scrapie in sheep, is not a prominent sign in BSE. BSE usually has an insidious onset and a slowly progressive clinical course of weeks to months.

Atypical BSE

Atypical BSE has usually been detected during surveillance testing of apparently-healthy cattle at slaughter, and by testing fallen cattle. Signs, when present, can be similar to classical BSE but, experimentally, have included mental dullness and amyotrophy.[14]

Exotic ungulate encephalopathy

Ataxia, tremor and weight loss are the most common of the various signs reported.[15]

Feline spongiform encephalopathy

As in BSE, changes in mental status (behaviour and temperament changes), hyperaesthesia and ataxia are the major signs of FSE in domestic and captive exotic cats.[15,16] In domestic cats, the ataxia particularly affects the hindlimbs, leading to a crouching gait. Other signs reported in domestic cats are nodding of the head, altered grooming, tremor, trismus, hypersalivation, polydipsia and polyphagia.

Chronic wasting disease

In deer and elk with CWD, signs reported include weight loss, emaciation, excessive salivation, unusual behaviour (including withdrawal from other animals, signs of depression, somnolence, aggression, repetitive activity, and teeth grinding), changes in posture and movement (including paralysis, dysphagia, head tremors, hind limb ataxia, and recumbency), polyuria, polydypsia, retention of winter hair coat, signs referable to aspiration pneumonia and, in subclinical and early clinical cases, sudden death after handling.[17,18] The clinical course varies from a few days to about a year, with most cases surviving from a few weeks to 3-4 months.[19] Signs may be more subtle and the clinical course more prolonged in elk than in deer.[19]

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.[20]

Human prion diseases

The various human prion 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 about 60 years, and there is rapidly progressive dementia with myoclonus, and death usually within a year.[5] In vCJD 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 linguistic group in the Eastern Highlands Province 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.

Epidemiology

Scrapie

Scrapie occurs in sheep, goats, and moufflon (a type of primitive sheep). Classical scrapie in sheep is associated with more than 20 strains of the scrapie agent, and with polymorphisms of the PrP gene at amino acid codons 136, 154 and 171. 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.

Scrapie is contagious and spreads naturally. There is a widespread presence of PrP^{Sc} within peripheral lymphoid tissues as well as in the CNS.[21] Transmission occurs from ewe to lamb in the period from parturition to weaning, and horizontal spread to unrelated sheep or goats also occurs, especially when parturition takes place in confined areas. Foetal membranes are a known source of infection,[21] and milk has recently been shown to be infectious to lambs.[22]

Atypical scrapie

In 1998, a new ('atypical') form of scrapie was found in sheep in Norway and designated 'scrapie Nor98' (other designations include 'Nor98', 'atypical scrapie' and 'atypical/Nor98 scrapie').[10,23] Since the introduction in 2002 of active surveillance for prion disorders of small ruminants using rapid immunochemical methods (testing of apparently-healthy sheep

and goats at slaughter, and of fallen stock), atypical/Nor98 scrapie has now been detected usually as single cases within sheep flocks throughout the European Union (EU), including regions free of classical scrapie,[9,24] and in the Falkland Islands,[25] the USA[26], Canada, New Zealand and Australia. While 80% sheep with classical scrapie are 2-5 years of age, atypical/Nor98 scrapie affects older animals (almost 60% are >5 years of age and 26% are >10 years).[27] Atypical/Nor98 scrapie in sheep is often associated with genotypes at PrP codons 136, 154 and 171 (ARR and AHQ) that are resistant to classical scrapie, and with another polymorphism, at codon 141, with phenylalanine (F) replacing leucine (L).[24] PrP^{Sc} is not detectible in peripheral lymphoid tissue.[10, 23] The distinct clinical, pathological and biochemical characteristics of atypical/Nor98 scrapie are maintained on experimental transmission and sub-passage in sheep.[13]

The diagnosis of atypical/Nor98 scrapie in a 12-year-old Swiss goat, found dead, may indicate a predilection for older animals also in this species.[28]

The origin and zoonotic risk of this rare condition are not yet known, but a spontaneous, non-contagious origin cannot be excluded.[23]

Bovine spongiform encephalopathy

BSE was first detected in British cattle in 1985[29] and caused a disease epidemic that was both novel and economically devastating. The diagnosis, since 1995, of cases of vCJD in Britain,[30] 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 compelling pathological and biochemical evidence that vCJD and BSE are caused by the same prion agent.[6]

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 4,000 in 1997 as the epidemic subsided. By the end of 1997 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 there is no evidence of horizontal spread, or of risk from semen, milk or through embryos.[31] There is no difference in breed susceptibility. Most cases are in cattle 4-5 years of age, but animals as young as 2 years may succumb.

Coincident with the BSE outbreak, putatively related animal TSEs were diagnosed in Britain:[32]

Exotic ungulate encephalopathy was diagnosed within the family Bovidae in captive antelope (nyala, gemsbok, eland, Arabian and scimitar-horned oryx, and greater kudu),[33,34] Ankole cattle and bison. These cases in zoo animals reflect exposure to the same BSE-contaminated feed that infected commercial British cattle.

Feline spongiform encephalopathy occurred within the family Felidae in domestic cats[16] and captive exotic cats (cheetah, puma, ocelot, tiger, lion and Asiatic golden cat).[3,15] These cases reflect the ingestion of feed containing the BSE agent. Infection of the exotic cats is attributed to the ingestion, in UK and European zoos, of BSE-infected carcase material from domestic cattle or other zoo animals.[2,3,15]

Atypical BSE

Since the introduction in 2001 of active surveillance for BSE of cattle using rapid immunochemical methods (testing of all apparently-healthy cattle aged 30 months or older at slaughter, and of all fallen stock older than 24 months), atypical forms of BSE have been detected across all continents where classical BSE is detected (throughout the European Union and also in Japan and North America).[31] Two types of atypical BSE, H-type and L-type, initially identified from Western immunoblot banding patterns,[35,36] have been further characterised by bioassay.[37] Most cases are in older animals (8-15 years of age).[35,38] The origin and zoonotic risk of these rare atypical forms of BSE are not yet known, but a spontaneous origin cannot be excluded.[39] A heritable, genetic mutation in the prion gene has been proposed as the aetiology of one case of H-type atypical BSE in the USA.[40]

Chronic wasting disease

Like scrapie, CWD of deer,[19] elk (wapiti)[19] and moose[41] is contagious and spreads naturally.[42,43] It is the only TSE known to affect free-ranging species.[19] CWD is spread by horizontal transmission through direct animal-to-animal contact and indirect exposure to the CWD agent via the contaminated environment, presumably by ingestion.[17,18] In CWD, abnormal PrP or infectivity is detectible not only within the CNS but also in blood, peripheral lymphoid tissues, pancreas, adrenal gland, skeletal muscle and myocardium.[18,42] CWD-infected animals may contaminate the environment via decomposing carcases or biological materials including saliva, blood, urine and faeces.[44,45] Unlike scrapie, maternal transmission does not appear to play an important role in CWD, and infectivity has not been identified in placentas of deer or elk.[18] Incubation periods typically range from 2-4 years and the disease has been reported in animals as young as 16 months and as old as >15 years.[18] Genetic polymorphisms in the PrP gene may affect CWD susceptibility, particularly at codon 225 (S/F) in deer and codon 132 (M/L) in elk.[43]

Of all the mammalian prion diseases, CWD is likely the most efficiently transmitted.[43] In captive cervids (deer and elk) in CWD-endemic facilities, prevalence of infection can be essentially 100%, while in free-ranging populations, prevalence is much lower but varies widely from <1% in deer and elk to about 30% in some dense deer populations.[18] CWD in moose has rarely been detected (three cases in 2005/2006 in Colorado, and one case in 2008 in Wyoming, all free-ranging).[41] The solitary social behaviour of moose may reduce the likelihood of CWD transmission.[41,42]

Despite reports in the popular press of humans affected by CWD, a study by the Centers for Disease Control and Prevention in the USA have not identified strong evidence for CWD transmission to humans.[46] In-vitro studies on conversion of human PrP by CWD-associated prions,[46] and CWD inoculation experiments in transgenic mice expressing human and cervid PrP, indicate that there is a substantial species barrier against CWD transmission to humans.[47]

Transmissible mink encephalopathy

TME transmission is linked to feed contaminated with a TSE agent of unknown origin, with possibilities including carcases of sheep or 'downer' cattle.[20,48] Attempts to experimentally infect mink by feeding scrapie-infected sheep or goat brain have failed.[20] L-type atypical BSE, which is phenotypically similar to TME when transmitted to ovine transgenic mice, is considered the most likely candidate if TME is caused by a TSE of cattle origin.[49]

Human prion diseases

Sporadic CJD is attributed to spontaneous somatic mutation in neuronal PrP, or rare stochastic conformational changes in expressed PrP.[5] Familial CJD, GSS and FFI are inherited diseases associated with mutations in the human PrP gene. Iatrogenic CJD is self explanatory. Kuru was transmitted by ingestion of, and possible conjunctival, nasal and skin contamination by, infected human CNS material during endocannibalistic ritual burial practices, which ceased in the late 1950s.

Most cases of vCJD are attributed to human ingestion of the BSE agent during the UK cattle epidemic; three recent cases are attributed to secondary transmission via blood transfusion from preclinical human vCJD cases (UK National CJD Surveillance Unit: http://www.cjd.ed.ac.uk/)

Occurrence and Distribution

Scrapie

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.

Atypical scrapie

In 2009, atypical/Nor98 scrapie was detected in one sheep brain from a consignment of sheep and goat brains sent from New Zealand to the European Union, for use as negative control materials for evaluating rapid tests for BSE and scrapie.[50] In 2010, a case of atypical/Nor98 scrapie was diagnosed in a sheep in Australia.

Bovine spongiform encephalopathy

BSE has never been diagnosed in Australia or New Zealand. Cases of BSE currently occur throughout most of Europe and have been detected in Asia (Japan) and North America.[31] http://www.oie.int/eng/info/en_esb.htm

Atypical BSE

Atypical BSE has not been detected in Australia or New Zealand.

Chronic wasting disease

CWD has been recognised in mule deer and Rocky Mountain elk since 1967 and 1979 respectively in captive wildlife research facilities in Colorado and Wyoming.[51] Between 1981 and 1990, CWD was seen in free-ranging elk, mule deer and white-tailed deer in these states and may have been present in some free-ranging deer populations for two decades or more before being detected.[19,52] With increased surveillance since the 1990s,[43] CWD has now been detected in farmed cervids (deer and elk), and/or free-ranging cervids (deer, elk and moose) in at least 18 states in the USA and two Canadian provinces (Saskatchewan and Alberta):

http://www.cwd-info.org/index.php/fuseaction/about.map

http://www.aphis.usda.gov/animal_health/animal_diseases/cwd/downloads/distribution_cwd.pdf

Outside of North America, CWD has been reported only from South Korea, and originated in a consignment of elk imported from Canada in 1997.[53]

Transmissible mink encephalopathy

TME is a rare disease of farmed mink that caused five outbreaks (four in Wisconsin and one in Idaho) on 11 ranches in the USA, between 1947 and 1985, and has also been reported in Canada, Finland, East Germany and Russia.[20,48]

Human prion diseases

Sporadic CJD, which comprises 85% of CJD cases, occurs worldwide at the rate of about one case annually per million people. Most of the 216 vCJD cases diagnosed to March 2010 occurred in the UK (169) and France (25). The annual incidence of UK deaths peaked at 28 in 2000. In Australia and New Zealand, sporadic CJD is the most common human prion disease; no case of vCJD has been detected. In Australia, iatrogenic CJD and familial clusters of CJD, FFI and GSS have been identified (Australian CJD Registry: http://ancjdr.path.unimelb.edu.au/).

Gross Pathology

There are no gross TSE lesions in the brain or lymphoid tissues. Gross changes secondary to CNS dysfunction include fleece damage in scrapie and aspiration pneumonia and weight loss in CWD.

Diagnostic Tests (General)

Diagnostic methods for the TSEs include clinical examination, histopathological CNS examination, PrP^{Sc} detection in CNS and lymphoid tissue, and experimental transmission (bioassay).[54]

In clinically-affected animals, microscopic CNS lesions are always present and histopathological examination, appropriately targeted, can have high diagnostic sensitivity and specificity. In classical BSE, with its consistent lesion profile,[55] 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, detected diagnostic neuroparenchymal vacuolation with a sensitivity of 99.6% and specificity of 99%;[56] however, recent unpublished evidence suggests a sensitivity as low as 95% for histopathological evaluation of the obex for vacuolar change.[31]

PrP^{Sc} can be detected in brain before histopathological lesions or clinical signs develop, and the available diagnostic tests for PrP^{Sc}, including immunohistochemistry (IHC), Western blot and rapid immunodiagnostic test methods (including ELISAs and a Western blot), are highly sensitive and specific.[21,31,54] The rapid PrP^{Sc} detection tests used for screening large numbers of samples in surveillance programs possess high diagnostic sensitivity and specificity. Most of these rapid tests can be automated, and results made available within a few hours.[54]

Detection of scrapie-associated fibrils (SAF) correlates well with the histological diagnosis of BSE but is not as sensitive as IHC and immunoblot methods.[31]

Serial protein misfolding cyclic amplification (sPMCA), an experimental procedure, has been used to detect PrP^{Sc} in various secretions, such as milk and urine, along with soil and water contaminated with PrP^{Sc} of putative animal origin.[22,44]

Guidance on Safety and Containment Requirements

TSE agents 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 (134-138^{o}C at 30 psi/203 kPa; holding time at temperature of 18 minutes for a single cycle, or 3 minutes for six separate cycles - some authorities advise holding times at temperature of at least one hour), and exposure to sodium hydroxide (1-2 M), or sodium hypochlorite (2-3% available chlorine) for at least one hour at 20^{o}C for working surfaces and overnight for equipment.[31]

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.

A recent publication has identified copper-hydrogen peroxide formulations as highly effective decontamination agents.[57] Further guidelines on TSE biosecurity are available.[58,59]

Part 2. Test Methods

Histopathology

Principle of the test

The hallmark of TSEs is the histological triad, in the CNS, of grey matter vacuolation, astrocytosis and neuronal degeneration (accompanied in some cases by cerebral amyloidosis). The distribution and severity of these changes depend on the TSE agent involved, the animal species affected and the duration of infection. For example, lesions are usually most prominent in the brainstem in classical scrapie, BSE and CWD; in the rostral areas of the brain, including the cerebral cortex, in FSE and TME; and in the cerebellum in atypical/Nor98 scrapie.

It is essential that diagnostic veterinary pathologists are prepared to detect a spongiform encephalopathy during routine histological examination of brains. Lesions in clinically-affected animals may be minimal or subtle, as in atypical/Nor98 scrapie.[23]

The hallmark neurohistological features of prion diseases are individually described under *Test procedure* and their expression in the specific TSEs of animals is detailed under *Interpretation of results.*

Histological examination has been supplemented or replaced by PrP detection methods for disease specific diagnosis of scrapie and BSE, particularly in large-scale, active surveillance programs in the Northern Hemisphere.[21,31,54]

Reagents and materials

Any species of animal with a 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. At a minimum, 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, ELISA, or as SAF by transmission electron microscopy. When a more comprehensive range of fresh brain samples is required for possible PrP^{Sc} screening, the

brain should be bisected by a sagittal, paramedian cut (0.5 cm off the median) and the smaller portion frozen (if testing is not to be done immediately after sampling).[31] The rest of the brain, after appropriate microbiological sampling, is fixed without distortion in 10% neutral buffered formalin for histological examination.

Whole brains for histopathological examination are fixed in 10% neutral buffered formalin for at least a week for sheep[21] and 2 weeks for cattle.[55] Shorter fixation periods of 3-5 days are adequate for brainstem samples.[21, 31]

For collection and examination of large numbers of samples in abattoirs for surveillance testing for scrapie and BSE, the brainstem can be removed via the foramen magnum without opening the skull, by means of a spoon-shaped instrument with sharp edges around the shallow bowl.[31]

Further details on collection and processing of samples are available.[21,31,58,60]

Selected formalin-fixed samples are routinely processed, paraffin-imbedded, sectioned and stained with haematoxylin and eosin for histopathological examination.

Test procedure

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,[61,62] has been adapted for study of naturally occurring TSEs in animals, including scrapie, BSE, FSE and CWD.[55,63-65] The 17 neuroanatomical profile areas for BSE (see Appendix 3) represent a range of brain levels; vacuolation severity for each profile area is scored on a scale of 0-4.[66]

In diagnostic specimens, two types of grey matter vacuolation should be sought: neuropil vacuolation (spongiform change, spongiosis), and neuronal (perikaryonal/nerve cell body) 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.[67] The neuropil vacuolation that is a feature of TSEs must be distinguished from 'status spongiosus' within grey matter, which is a nonspecific 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.[68,69] To avoid confusion in this document, the term 'status spongiosus', narrowly defined above, is not applied to other forms of CNS vacuolar change, such as 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.

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 of nerve cell bodies, often produce ballooned neurons with a narrow rim of cytoplasm.[29] Vacuolated neurons also occur in normal brains of sheep, goats and cattle[70] 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[29,70-72] and dorsal root ganglia (GA Wells, personal communication), and may occur singly in the reticular formation of the medulla and at other sites.[71] 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[73] and in young Rottweiler dogs.[74]

Astrocytosis

In routinely-stained CNS sections, astrocytosis is most marked in classical 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 (e.g. 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.[75,76] 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 techniques.[66]

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 vCJD, but are less frequent in other TSEs. They are found in about 5% of CJD cases, and in <5% of most animal TSEs. 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.[76] Amyloid plaques have been reported in the thalamic neuropil of goats with scrapie,[8] and of cattle with BSE,[70] L-type BSE,[77] and in the cerebral cortex and diencephalon of many deer with CWD, but have not been reported in elk with CWD[51] or in TME. 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).

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, but its diagnostic significance is unclear.[55,71,78,79] Similar vacuolation was seen in the white matter radiation from the dorsolateral thalamus in sheep with neurological signs.[80] 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.[81] 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,[82] and multifocal necrotising encephalopathy in Limousin, Simmental and Angus calves.[83] 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.[84]

Quality control aspects

All laboratories involved in histopathological examination of animal brains for national TSE surveillance should participate in proficiency testing programs for TSE histopathological interpretation, where they exist.

Interpretation of results

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 scrapie,[75] and may be obscured by autolysis and tissue disruption during processing.[56] 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 patterns of neuropil vacuolation described in sheep with scrapie comprise various degrees of rostral extension from the obex to the cerebral cortex.[76] 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.[8,76] Neuronal degeneration, producing pyknotic, dark neurons, particularly in areas rostral to the midbrain, is a consistent feature of scrapie in sheep[76] and goats.[8]

Atypical scrapie

Neuropil vacuolation predominates over neuronal vacuolation in atypical/Nor98 scrapie. In contrast to classical scrapie, vacuolation is most prominent in the molecular layer of the cerebellum and in the cerebral cortex, is minimal in the brainstem, and is usually not detectible at the obex.[10] This lesion profile reflects the distribution of accumulated PrP^{Sc} within the brain.[13] With immunohistochemical (IHC) methods, PrP^{Sc} staining is conspicuous in the cerebellum, but is less prominent at the obex, where it is absent from the dorsal vagal nucleus but is commonly present (although restricted and frequently minimal) in the nucleus of the spinal tract of the trigeminal nerve, and is sometimes present in the reticular formation, ambiguous nucleus, and as heavy and pronounced globular staining of the white matter tracts.[9,23]

In a case of atypical/Nor98 scrapie reported in a Swiss goat, neuropil vacuolation and astrocytosis were detected in the rostral areas of the brain only (including midbrain, thalamus, basal ganglia and cerebral cortex) but not in the cerebellum or medulla, and neuronal vacuolation was not detected. In this case, PrP staining by IHC correlated with neuropil vacuolation, with the exception of mild immunolabelling detected in the cerebellar cortex and in the spinal tract nucleus of the trigeminal nerve.[28]

Bovine spongiform encephalopathy

Neuropil vacuolation predominates over neuronal vacuolation, and is the most striking and diagnostically significant change in BSE.[66] 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.[55] 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.[55,66] 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.[56] Neuronal vacuolation is most prominent in the vestibular nuclear complex and red nucleus in BSE.[70] 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.[87]

Exotic ungulate encephalopathy

There are differences in the relative severity of involvement of certain neuroanatomical nuclei compared to BSE.[32] For example, in the nyala case, neuropil and neuronal vacuolation in the dorsal vagal nucleus were more intense than in BSE.[88]

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.[16] Neuronal

vacuolation has a caudal predilection, affecting the dorsal vagal nucleus, raphe nucleus, vestibular nuclear complex, red nucleus, and the occasional neuron in the spinal cord.[16] In a case of FSE in a puma, neuronal vacuolation was the most striking change.[89] Astrocytosis and an increase in numbers of rod-shaped microglia also occur in FSE.[16] In domestic cats with FSE, there was also vacuolation of white matter mainly in the medulla, associated with axonal degeneration, particularly in the pyramidal tracts.[16] A puma with FSE also had some axonal degeneration in spinal cord white matter.[89] A cheetah with FSE had widespread axonal degeneration and demyelination of all tracts in the spinal cord,[2] and an Asiatic golden cat with FSE had widespread white matter vacuolation of the corpus callosum, internal capsule, thalamus and brain stem (as well as widespread, mild, neuropil vacuolation of deep laminae of the cerebral cortex, midbrain and brainstem nuclei, with occasional small single neuronal vacuoles).[3]

Atypical BSE

In many reports only brainstem was available for assessment, with some cases having vacuolation consistent with classical BSE and others not. In the original two Italian L-type BSE cases, which occurred in 11- and 15-year-old animals, vacuolation was not consistently found in the brainstem, although PrP-amyloid plaque-like deposits were detected in the thalamus, pyriform cortex and olfactory lobe.[77]

Chronic wasting disease

Neuropil vacuolation is the most striking change in deer and elk with CWD and is usually accompanied by neuronal vacuolation.[51] 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. 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 a 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 (producing 'dark' neurons) in the cerebral cortex, and neuronal loss in the thalamus.[51] Amyloid plaques are detected in most clinically-affected white-tailed deer and in a few mule deer, but are not obvious in elk.[19] In elk with CWD, there can be mild axonal degeneration in the cerebrum and cerebellum.

Transmissible mink encephalopathy

Neuropil vacuolation, neuronal degeneration (producing '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.[20] Neuronal vacuolation is much less common than neuropil vacuolation, and is found mainly in the brainstem.

Detection of Disease-Specific Forms of Prion Protein (PrP)

Details of current test procedures approved by the World Organisation for Animal Health (OIE) for PrP^{Sc} detection (including the commercially-available rapid tests) are available from the website of the Veterinary Laboratory Agency (VLA), Weybridge UK, and are not included in this document. VLA is the European Community TSE Reference Laboratory[58] and is also an OIE TSE Reference Laboratory:[60]

http://www.defra.gov.uk/vla/science/sci_tse_rl.htm

http://www.defra.gov.uk/vla/science/sci_tse_oie.htm

Details of the application and limitations of these tests are available in the chapters on scrapie and BSE in the OIE Manual of Diagnostic Tests and Vaccines 2008:[21,31]

http://www.oie.int/eng/normes/mmanual/A_summry.htm

http://www.oie.int/eng/normes/mmanual/2008/pdf/2.07.13_SCRAPIE.pdf

http://www.oie.int/eng/normes/mmanual/2008/pdf/2.04.06_BSE.pdf

The tests for PrP^{Sc} in CNS samples are currently carried out at the Australian Animal Health Laboratory, Geelong, Australia and at the Investigation and Diagnostic Centre (IDC), Wallaceville, New Zealand.

In sheep, palatine tonsil, nictitating membrane, superficial lymph nodes and rectal mucosa have been sampled for preclinical detection of PrP^{Sc} in lymphoreticular tissue for classical scrapie diagnosis.[21] In CWD, as in classical scrapie, lymphoid tissue as well as CNS tissue can be tested for PrP^{Sc}.[54]

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.

Immunohistochemical (IHC) methods

Immunostaining of formalin-fixed or unfixed, cryostat preparations of CNS tissue identifies the topographical and cellular localisation of accumulated PrP^{Sc}. Pretreatment steps to unmask PrP epitopes in formalin-fixed tissue sections include hydrated autoclaving, and proteinase K or formic acid treatment (the last also decontaminates TSE-positive tissue). Sections are then stained in an indirect immunoperoxidase test using monoclonal antibody to a PrP epitope. The IHC techniques can be applied to mildly autolysed tissue that is unsuitable for routine histological evaluation.

Western blot methods

Immunoblot techniques have similar diagnostic sensitivity to the IHC techniques, and both are methods of choice for confirmation of a scrapie, BSE or CWD diagnosis.[21,31,54] The original 'OIE Western immunoblot' method (also known as the 'OIE-SAF Western blot') for PrP^{Sc} detection uses a relatively large amount of CNS tissue (4 g), detergent extraction, lengthy ultracentrifugation steps to concentrate any PrP^{Sc} and, finally, treatment with proteinase K to eliminate PrP^{C}.[21] Current immunoblot techniques are less time-consuming and less costly. They use less CNS or lymphoid tissue (up to 0.5 g), use phosphotungstic acid or other chemicals to precipitate PrP^{Sc} and, due to the use of appropriate combinations

of homogenization buffers and antibody, do not need the centrifugation steps.[54] Some are commercially available.

Proteinase K (PK) digestion cleaves approximately 70 N-terminal amino acids from the extracted PrP^{Sc} (32-35 kDa), and results in a Western blot with typically three bands, corresponding to diglycosylated (27-30 kDa), monoglycosylated (23-25 kDa) and unglycosylated (18-21 kDa) fragments of digested PrP^{Sc}. Deglycosylation of the digested PrP^{Sc} using PNGase enzyme typically results in a Western blot with a single unglycosylated band. The molecular weight and consequent electrophoretic mobility of the unglycosylated band reflect the site of PrP^{Sc} cleavage by proteinase K and, together with the relative proportions of the three glycoforms in the blot, can be characteristic for a particular prion disease. Immunoblotting has the potential to distinguish scrapie strains on the basis of these glycosylation patterns, but this is not yet a routine operational method.

A hybrid Western blot method with double antibody discrimination can distinguish between scrapie and BSE. The higher sensitivity of the BSE agent to PK digestion, results in loss of a larger N-terminal amino acid fragment, including an amino acid sequence that is retained intact in digested scrapie samples and is detectible with monoclonal antibody P4 in scrapie blots (but not in BSE blots).[54,60]

Atypical/Nor98 scrapie has distinctive features on Western blot; there are multiple bands, including a distinctive lower band with a molecular mass <15 kDa and, following PNGase treatment, there are three different unglycosylated bands (in contrast with the typical single unglycosylated band in classical scrapie and other TSEs).[23]

Rapid test methods

A number of ELISAs, and a Western blot technique, provide rapid PrP^{Sc} detection methods that are used for screening large numbers of samples in scrapie, BSE, and CWD surveillance programs.[21,31] These tests have been comprehensively evaluated for scrapie,[90,91] BSE[92] and CWD[93] and possess high diagnostic sensitivity and specificity. Most of these rapid tests can be automated, and results made available within a few hours. Western blot and rapid ELISA tests can be applied to autolysed tissue, but Western blotting is more reliable.[94]

Scrapie associated fibrils (SAF)

SAF are disease-specific, ultrastructural TSE markers that comprise PrP^{Sc}. They are demonstrated in detergent- and proteinase K-treated extracts of unfixed CNS tissue by negative staining using transmission electron microscopy.[95] With modification, the method can be applied successfully to formalin-fixed tissue.[31] SAF can be detected in autolysed tissue.

Bioassay

Detection of a TSE agent by transmission tests in sheep, goats, cattle or mice, is a lengthy procedure and, therefore, impractical for routine diagnosis. It has been part of the Australian and New Zealand quarantine requirements for importation of sheep and goat embryos from scrapie-affected countries. Transgenic mice over-expressing the human, sheep, deer, elk, or cattle PrP gene have the potential to increase mouse bioassay sensitivity (due to their genetic closeness to a specific natural host) and to reduce duration of the incubation period,[54] but such bioassays are research rather than routine diagnostic tools.[37]

Other tests

The detection of protein markers of neurodegeneration, including apolipoprotein E (ApoE), the 14-3-3 protein and S-100 in cerebrospinal fluid have not proved useful for diagnosis of preclinical cases of BSE.[31]

sPMCA is an in vitro technique that amplifies minute amounts of PrP^{Sc} to detectable levels by expediting conversion of PrP^{C} to PrP^{Sc}.[22,44] This procedure, although not yet validated for routine testing or confirmation of results, nonetheless offers promising opportunities to test products and samples in a manner not previously possible.

Australian and New Zealand diagnostic standard for TSE exclusion

The diagnostic standard for TSE exclusion involves histopathological examination of transverse sections of the brainstem at three standard sites: the obex, caudal cerebellar peduncles, and midbrain (See Appendices 1, 2 and 3).

In Australia, if the histopathological evaluation is not clearly 'TSE Histopathology Negative' and there is no alternative diagnosis for the neurological signs, CNS specimens are sent to the Australian Animal Health Laboratory (AAHL), Geelong, Victoria for PrP testing. In New Zealand, if a 'TSE Histopathology Negative' diagnosis cannot be given, all fresh and fixed tissues, slides and blocks are referred to the Investigation and Diagnostic Centre (IDC), Wallaceville.

Evaluation of the standard sites can be part of the routine histological examination of any animal brain, but is required for TSE exclusion in cases of progressive neurological disease. The Australian National TSE Surveillance Program targets cattle between 30 months and 9 years of age and sheep 18 months and older. The New Zealand TSE Incentive Surveillance Program targets cattle between 30 months and 9 years of age and sheep, goats and deer 2 years and older.

All laboratories involved in the histopathological examination of animal brains for national TSE surveillance should participate in proficiency testing programs for TSE histopathological interpretation, where they exist.

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Appendix 1

Australian and New Zealand diagnostic standard for TSE exclusion

Exclusion of TSE should be part of the examination of the brain from any species of animal with a progressive neurological disease.

Specimens

The brain, with brainstem intact, is removed from the skull as soon as possible after death. Unfixed samples (3-10 g) of medulla caudal to the obex**,** and/or cervical spinal cord**,** plus cerebellar cortex from sheep and goats, are frozen for possible testing for prion protein (PrP). The rest of the brain, after appropriate microbiological sampling, is fixed, without distortion, in 10% neutral buffered formalin for histopathological examination.

Standard brain sites for histopathological examination

Transverse sections of the brainstem are made at the following three standard sites for TSE exclusion.

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

Evaluation of these sites can be part of the routine histopathological examination of any animal brain, with examination extended to other brain sites (e.g. cerebellum, thalamus, basal ganglia and cerebral cortex) as required. In sheep and goats, cerebellum should be examined to facilitate differentiation of scrapie and atypical/Nor98 scrapie.

Histopathological changes in TSE

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

- Vacuolation of grey matter neuropil (spongiform change), and/or neurons.
- Astrocytosis.
- Neuronal degeneration (producing ‘dark’ neurons) and/or neuronal loss.
- Amyloidosis.

Histopathological diagnosis and reporting *

*TSE Histopathology Positive**

Characteristic vacuolation of grey matter neuropil (spongiform change) and/or neurons is present, usually with a bilaterally symmetrical distribution. Astrocytosis and neuronal degeneration 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 Histopathology Inconclusive*

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

TSE Histopathology Unsuitable*

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

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

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

Further testing is required if the histopathological TSE evaluation is not clearly 'TSE Histopathology Negative' and there is no alternative diagnosis for the neurological signs.

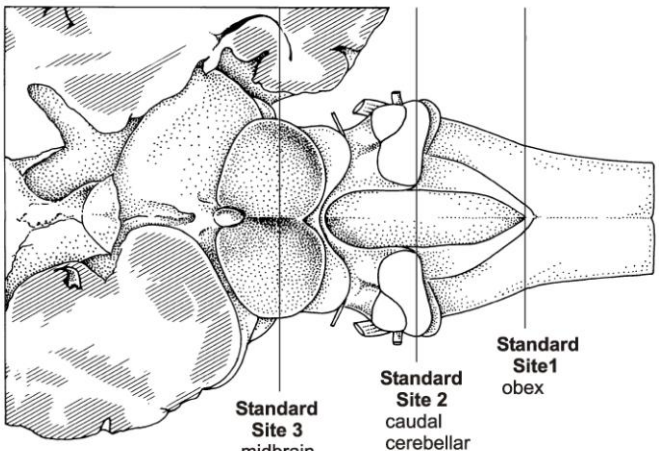
CNS specimens for PrP testing are sent to the Australian Animal Health Laboratory, Geelong (AAHL), Victoria, Australia or the Investigation and Diagnostic Centre at (IDC) Wallaceville in New Zealand.

Accumulated abnormal PrP is detected in formalin-fixed CNS tissue by immunohistochemical (IHC) test, or in extracts of the unfixed CNS sample by immunoblot or ELISA test, or as disease-specific 'scrapie associated fibrils' (SAF) by transmission electron microscopy. Bioassay by transmission tests in ruminants or mice is available but is a lengthy procedure.

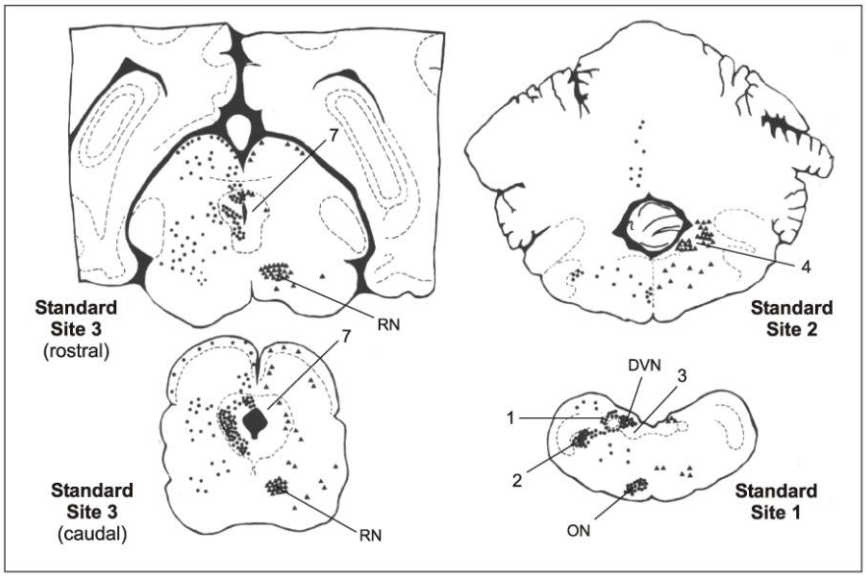
****Recommended wording to conclude the four categories of TSE reports.***

Australian and New Zealand Standard Diagnostic Procedure
Transmissible Spongiform Encephalopathies

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.

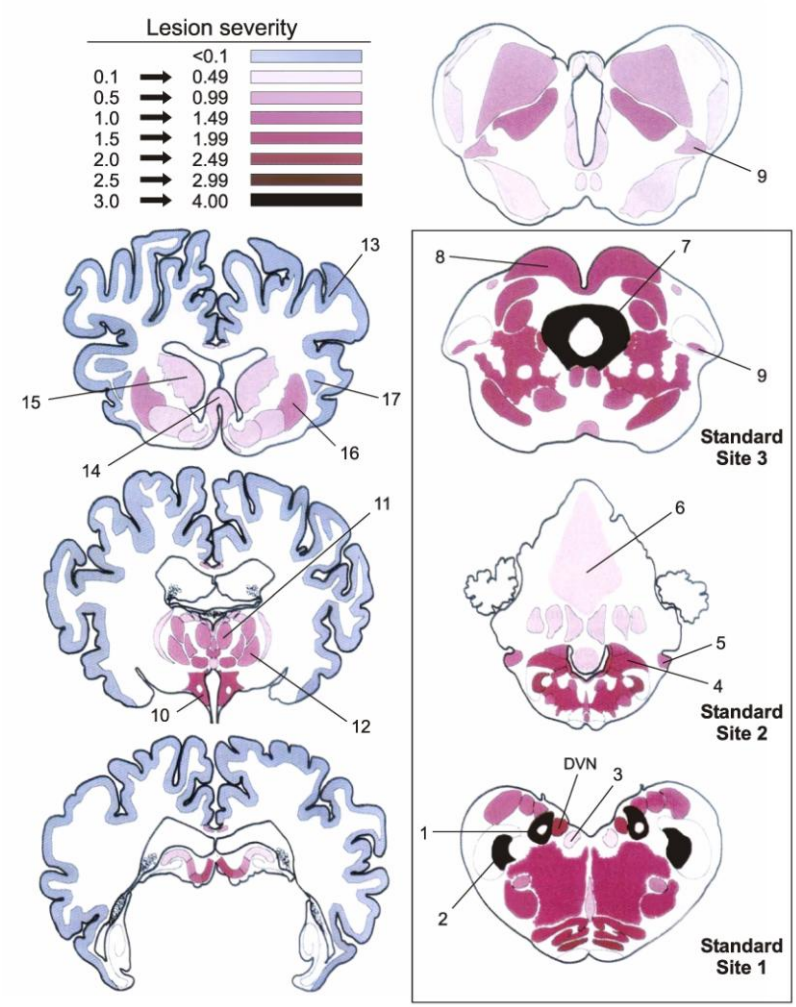
Vacuolation in bovine spongiform encephalopathy (BSE):
- • (left half) = Grey matter neuropil vacuolation (spongiform change).
- ▲ (right half) = Neuronal vacuolation.

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

Australian and New Zealand Standard Diagnostic Procedure
Transmissible Spongiform Encephalopathies

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