Diagnosis of transmissible spongiform encephalopathies in animals: a review

Dolores Gavier-Widén, Michael J. Stack, Thierry Baron, Aru Balachandran, Marion Simmons

Abstract. Transmissible spongiform encephalopathies (TSEs) in animals include, among others, bovine spongiform encephalopathy (BSE), scrapie, chronic wasting disease, and atypical forms of prion diseases. Diagnosis of TSEs is based on identification of characteristic lesions or on detection of the abnormal prion proteins in tissues, often by use of their partial proteinase K resistance property. Correctly sampling of target tissues is of utmost importance as this has a considerable effect on test sensitivity. Most of the rapid or screening tests are based on ELISA or Western immunoblot (WB) analysis, and many are officially approved. Confirmatory testing is normally performed by use of histologic examination, immunohistochemical analysis, certain WB protocols, or detection of prion fibrils by use of electron microscopy (scrapie-associated fibril). The discriminatory methods for diagnostic use are mostly based on WB technology and provide initial identification of the prion strain, particularly for differentiation of BSE from scrapie in small ruminants. Definitive prion strain characterization is performed by use of bioassays, usually in mice. A burgeoning number of transgenic mice have been developed for TSE studies. Development of new tests with higher sensitivity and of more reliable diagnostic applications for live animals tested for food safety reasons is a rapidly developing field. Ultimately, the choice of a test for TSE diagnosis depends on the rationale for the testing.

Key words: BASE; BSE; CWD; Nor98; prion; scrapie; TSE; transmissible spongiform encephalopathy.

Introduction

Definition

Transmissible spongiform encephalopathies (TSEs) or prion diseases are a group of infectious neurodegenerative diseases that affect humans and animals. They include bovine spongiform encephalopathy (BSE), scrapie, chronic wasting disease (CWD), transmissible mink encephalopathy, feline spongiform encephalopathy, and others. In humans, among others, Creutzfeldt-Jakob disease (CJD) with the sporadic (sCJD), variant (vCJD), iatrogenic and familial subtypes exist. The cause of TSEs, although still debatable, is widely accepted to be a novel type of infective agent, a transformed host protein called a prion (proteinaceous infectious particle).

History

The first naturally acquired TSE to be recognized was scrapie, with descriptions dating back to the 18th Century. Today it occurs in small ruminants in many countries, with the notable exceptions of Australia and New Zealand. Transmissible mink encephalopathy was at first recognized as a food-borne disease of ranch-raised mink in the USA, and was, thereafter, detected from time to time in several other countries. Chronic wasting disease was first recognized as a clinical syndrome of unknown etiopathogenesis in cervids in 1967 in a captive mule deer sourced from free-ranging populations in Colorado (USA). In 1978, a similar syndrome was identified in Wyoming (USA), and in the same year spongiform encephalopathy was observed in affected animals from the Colorado facility. Today, CWD occurs in free-ranging and farmed cervids in several locations in the USA and in Saskatchewan and Alberta in Canada. Bovine spongiform encephalopathy was first identified in the UK in 1986, and was propagated in cattle by recycling meat and bone meal (MBM) from infected cattle. This resulted in a large-scale epidemic, which has to date affected more than 182,000 cattle. With the advent of rapid screening tests, and the imposition of standardized active surveillance regulations throughout the European Union (EU), BSE was identified in many countries that were previously assumed to be free of the disease. More than 3,800 cases have been detected outside the UK, including those in 13 European member countries, Japan, Canada, and the USA.

The Prions

The cellular prion protein (PrPc) is a glycosylphosphatidylinositol (GPI)-linked copper-binding membrane protein of approximately 220 amino acid residues typically in α-helical conformation. Prions are
normal cell surface proteins of neurons and other cells, and are highly conserved across diverse species including mammals, reptiles, fish, and amphibians, but their function is not yet clear. They are encoded by a unique Prnp gene. The pathologic isof orm of PrPC, called PrP scrapie (PrPSc), although the terms PrPCWD and PrPBSE also are used to specify the source. It is an abnormal, relatively protease-resistant isof orm of the PrP protein, rich in β-pleated sheets. The PrPC is converted into the abnormal disease-associated form through a process of conformational change, whereby some of its α-helical structure is replaced by β-sheets; this form can adopt a fibrillar aggregated structure that is characteristic of many of the deposits found in the brain of TSE-affected species. This posttranslational modification of PrPC into the abnormal, infective isof orm, PrPSc, is believed to be the principal molecular basis of prion diseases.

In 1982, the “protein only” hypothesis proposed that PrPSc was the cause of TSE and was infective on its own, and that it replicated by interacting with the host PrPC so that the normal cellular protein would undergo a conformational conversion into PrPSc. This proteinase kinase (PK)-resistant PrPSc accumulated in the tissues; it could not be degraded as is the normal PrPC and became the most effective marker of prion infection. The fraction resulting from the treatment of PrPSc with PK is termed PrPres (res meaning resistant), and most diagnostic methods are based on its detection. The PrPSc cannot be inactivated by use of formalin, is highly resistant to traditional sterilization methods such as autoclaving and disinfectants, and has an extraordinary environmental persistence. The BSE agent, like all of the human TSE agents, is categorized as a hazard group-3 pathogen, but as there is no evidence of airborne transmission, full containment level 3 is not required. Similarly, according to the US Center for Disease Control, BSE laboratory work can be conducted under biosafety level 2 or 3, depending on the risk of the material to be examined.

Concern for human infection: history and rationale for testing for TSE

The finding that a new type of CJD called variant or vCJD was associated with a form of infective agent that was indistinguishable from that of BSE was alarming. More than 150 cases of vCJD have been diagnosed in the UK and a few in other countries. The public fear has been compounded by a report of likely iatrogenic vCJD transmission via blood products. The BSE concern prompted large-scale testing. Today, European regulations require rapid screening for BSE on all cattle aged more than 30 months that are slaughtered for human consumption. More than 30 million cattle have been tested since 2001 (http://europa.eu.int/scadplus/leg_lvb/f83002.htm).

As knowledge in the field of TSE expands, new uncertainties arise, and the host range for BSE has been redefined. Results of several experiments have indicated that sheep and goats orally inoculated with BSE develop a disease clinically and pathologically indistinguishable from scrapie. It also is well established that small ruminants received feed supplements containing contaminated MBM over the time period that cattle were exposed. This finding raised the hypothetical possibility that cases diagnosed as scrapie could actually be BSE in infection, and that endemc scrapie may mask BSE infections in the sheep population. This further raised questions about the safety of consuming sheep meat. Subsequently, obligatory large-scale active surveillance of small ruminants was implemented in the EU in 2002, initially applying rapid tests that had only been validated specifically for cattle. In January 2005, a goat that was test positive for TSE in France in 2002 was confirmed to be BSE positive. This was the first confirmed case of natural BSE in a small ruminant. However, uncertainty about potential susceptibility of humans to BSE from species other than bovine remains.

Even less is known about the infectivity for humans and other animals of the newly recognized atypical strains of TSEs in cattle and sheep. The BSE experience, together with the unknowns about TSE, has resulted in renewed concerns about a possible food-borne zoonotic role for other prion diseases of animals, such as CWD. The concern over CWD in North America has increased with the discoveries of CWD in increasing numbers in wild cervids in several states in the USA and in Saskatchewan (Canada). The incidence of CJD, particularly among hunters, their relatives, and other venison consumers has periodically been questioned with a possible link to CWD, but has not been confirmed as a new entity or any different from classic CJD. It is reassuring that, until today, human TSE infection acquired through consumption of venison or sheep meat has not been documented.

Pathogenesis and pathologic characteristics of TSEs

Animal TSEs are principally acquired by the oral route. In the early stages, PrPSc crosses the mucous membranes and is detected in the tonsils, Peyer’s patches, and lymph nodes draining the alimentary tract. This early lymphoid tropism has been observed experimentally in CWD as early as 6 weeks after infection and in natural scrapie in lambs from 3 months of age onward. A prolonged replication
phase of the pathogenic PrP of months to years takes place in the lymphoreticular system (LRS) including the spleen and lymph nodes. However, in BSE and in some scrapie cases, there is little involvement of the LRS.42,101,104 Thereafter, PrPSc is detected in the brain where it progressively accumulates, causing fatal neurodegenerative changes. The mechanisms of transit of the PrPSc from the gut or tonsils into the brain are not yet clear. The hematogenous and retrograde axonal routes, involving fibers innervating lymphoid tissues or the autonomic nervous fibers of the digestive tract, have been implicated.3,82

The TSEs are characterized by 2 types of vacuolar changes limited to the gray matter. One is vacuolation of neuronal processes, observed as spongiosis or vacuolation in the neuropil, and the other type is vacuolation of neuronal bodies, observed as single or multiple vacuoles in the perikarya of neurons.85,87,100,102,109 Accumulation of PrPSc in neurons and glial cells in the brain is the hallmark of TSE, and can be detected at earlier stages than can vacuolar changes.42,85,87 Other changes that are characteristic of some human prion diseases are variably observed in some of the animal TSEs.105 They include neuronal death or loss, proliferation of astrocytes, and formation of amyloid plaques. Primary vacuolation of the white matter is not a lesion specific for TSEs. Inflammatory responses do not appear to be mounted against the pathogenic PrP; most likely because it is not perceived as foreign by the immune system. The mechanisms of brain damage caused from the accumulation of PrPSc have not been fully elucidated. Remarkably, histologic lesions are not observed in lymphoid tissues in any of the TSEs, even with marked PrPSc accumulation.

Biochemical properties of PrPSc

Biochemically, the properties of PrPSc appear to be determined by the 3-dimensional shape of the molecule, changes to which could result in different forms or “strains.” In all TSEs, the important biochemical parameters are related to the 3 protein bands that are the molecular signature, visible by use of WB analytic methods. These are the 3 bands that correspond to antibody detection of a diglycosylated region of the PrPSc molecule, a monoglycosylated region, and an unglycosylated region, at around 29, 25, and 20 kD, respectively.88 Variations in the amount of signal detection for each band, the molecular weight position on the gel, and immunoreactivity with various monoclonal or polyclonal antibodies are now the methods used to try and differentiate between the various types of PrPSc.90

Features of animal TSEs

**Bovine spongiform encephalopathy (BSE)**

*Host range, clinical signs of disease, and epidemiology.* Bovine spongiform encephalopathy is unique among the TSEs in that it has extensively crossed the species barrier. Besides the already mentioned human form, vCJD, natural BSE infection has occurred in 7 species of the family Bovidae, in 4 Felidae, and in 4 nonhuman primates in zoological collections.49,81,110 Feline spongiform encephalopathy also has been documented to be a BSE infection of domestic cats.11,111 The BSE incubation period in cattle is approximately 4 to 5 years. Clinical signs of disease include behavioral abnormalities, hyper-reactivity, apprehension, gait abnormalities, weakness, loss of body condition, and recumbency.30 Bovine spongiform encephalopathy is transmitted by the dietary route. Evidence of horizontal or vertical transmission has not been identified; nonetheless, there is an increased risk for BSE in the offspring of clinical cases.106

*Histopathologic changes and PrPSc distribution.* The most characteristic histologic change in BSE consists of bilaterally symmetric vacuolation of the gray matter neuropil (spongiosis), with a particular preference for certain neuroanatomic locations.100,105 In BSE, the distribution of lesions is dependent on the host species. In cattle, areas most consistently and severely affected are the solitary tract nucleus, the spinal tract nucleus of the trigeminal nerve, and the central gray matter of the midbrain.100,105 The neuropil vacuolation of the target nuclei is considered to be pathognomonic for BSE (Fig. 1). Intraneuronal vacuolation is also observed in BSE, but this feature alone, in the absence of neuropil vacuolation, is not confirmatory since vacuolated neurons, particularly in certain locations, such as the red nucleus, may be an incidental finding in cattle.31 In BSE, immunohistochemical (IHC) analysis consistently reveals PrPSc accumulation in the brain, with distribution similar to, but often more widespread than neuropil vacuolation. Several morphologic types of PrPSc deposition can be present in the brain of an individual: intraneuronal, perineuronal, linear, fine punctate, and coarse particulate.105 In cattle, PrPSc cannot readily be detected in tissues outside the central nervous system (CNS), although limited involvement of the Peyer’s patches in the distal portion of the ileum has been documented in experimentally induced and naturally acquired cases of BSE.94

*Biochemical properties of PrPSc.* The main BSE signature, using antibodies directed at the core of the PrP molecule (e.g., monoclonal antibody [MAb] 6H4) is a dominant diglycosylated band, followed by a less intense monoglycosylated band, and finally, the weakest signal from the unglycosylated protein band. The molecular mass of the unglycosylated band is usually lower than that seen for scrapie controls.90

**Scrapie (typical scrapie)**

*Host range, clinical signs of disease, and epidemiology.* Scrapie naturally affects sheep and goats. The
Figure 1. Photomicrographs of a section of the medulla oblongata (obex) from a cow with bovine spongiform encephalopathy (BSE). The presence of typical neuropil vacuolation in the solitary tract nucleus is a diagnostic feature of BSE. (Haematoxylin & eosin; 400×). Courtesy of Gerald Wells, Crown Copyright. a. Mild neuropil vacuolation (minimal lesions). b. Severe neuropil vacuolation (advanced lesions).

disease develops most often in sheep 2 to 5 years of age, and commonly has an incubation period of more than 1 year. Clinical signs of disease include behavioral changes, ataxia, pruritus, weakness, recumbency, and loss of body condition. Scrapie is maintained and disseminated by horizontal transmission. The PrPSc is demonstrated in the placenta of infected ewes, which is a source of infection for the offspring and other sheep in the flock, as well as for goats.

Genotypes. The susceptibility of sheep to scrapie has been shown to be heavily dependent on the PrP genotype of the host. The main polymorphic PrP gene sites known to influence susceptibility are codons 136 (A or V amino acid alternative), 154 (R or H) and 171 (Q, R or H). The VRQ (V136R154Q171) allele is linked to highest susceptibility; the AHQ allele is related to increased resistance, whereas ARR confers the highest resistance. Other alleles are intermediate.
Diagnosis of TSE: review

Figure 2. Photomicrograph of a section of the brain from a sheep with scrapie. Severe neuronal and neuropil vacuolation are characteristic of an advanced case. (Haemotoxylin & eosin; 200×). Courtesy of Gerald Wells, Crown Copyright.

bility to scrapie also has been documented to be influenced by the isolate of scrapie agent, the flock, and the breed. Programs for selective breeding for scrapie resistance have been implemented in many countries as part of a control strategy (www.defra.gov.uk/nsp), and current EU control policies advocate culling on a selective genotype basis.

Histopathologic changes and PrPSc distribution. Neuronal and neuropil vacuolation are characteristic of scrapie (Fig. 2). Lesion distribution in the brain is variable according to prion strain and genotype of the sheep, but all typical scrapie cases show accumulation of PrPSc in the medulla oblongata. Several immunohistochemical CNS patterns in scrapie have been described. Extracellular types are: astrocyte-associated (stellate, subpial, subependymal, and perivascular), neuropil-associated (linear, fine punctuate, coarse particulate, coalescing, and perineuronal), ependymal cell-associated (supraependymal) and endothelial cell-associated (vascular plaques). The intracellular types are intraneuronal and intragliaial (Community Reference Laboratory [CRL] [http://www.defra.gov.uk/corporate/vla/science/science-tse-rl-intro.htm]).

In scrapie, results of immunohistochemical (IHC) analysis indicate accumulation of PrPSc in the LRS, such as tonsils, lymph nodes, spleen, and gut-associated lymphoid tissue, as well as placenta and peripheral nervous system (PNS). In the lymphoid tissues, the PrPSc accumulates in secondary follicles associated with tingible body macrophages (TBM) and follicular dendritic cells. The peripheral distribution of PrPSc is influenced by strain of scrapie and by genotype (it is not detected in sheep of the VRQ/ARR genotype).

Biochemical properties of PrPSc. Several distinct strains of scrapie in ovine brain-inoculated mice have been described on the basis of incubation period and lesion profiles. For natural, classic scrapie cases, the biochemical properties exhibited by WB appear to be uniform, irrespective of geographic origins, breed, or genotype. Scrapie samples show the usual characteristic 3 protein bands, also described for BSE when MAb 6H4 to the core of the PrP protein is used for detection. However, the molecular weight of the unglycosylated band for scrapie samples is higher than that for BSE. Also in scrapie, the same 3 bands are detected by use of an MAb P4 to a specific N-terminal sequence of the protein, whereas no bands are detected in BSE samples.

Chronic wasting disease (CWD)

Host range, clinical signs of disease, and epidemiology. Chronic wasting disease is naturally acquired in 3 species of the family Cervidae: mule deer (Odocoileus hemionus hemionus), white-tailed deer (Odocoileus virginianus), and Rocky Mountain elk (Cervus elaphus nelsoni). It is possible that subspecies of these, including the black-tailed deer (Odocoileus hemionus columbianus), are naturally susceptible. Susceptibility to CWD also appears to be related to polymorphisms in the Prnp. Clinically, CWD is characterized by behavioral changes and loss of body condition, which are milder and more chronic in elk than in deer. In Canada, a clinical diagnosis of CWD is considered in cervids over 16 months of age that are manifesting any of the following: weight loss, excessive salivation, unusual behavior (including withdraw-
al from other animals, signs of depression, somnolence, and aggression) signs of neurologic dysfunction (including paralysis, dysphagia, head tremors, hind limb ataxia, or recumbency), polyuria/polydypsia, retention of winter coat, and signs referable to aspiration pneumonia. Chronic wasting disease is contagious and is transmitted laterally. Pastures and paddocks apparently become contaminated by excreta and saliva, even prior to the onset of clinical disease, and have been incriminated as a source of infection. In clinically affected deer, the vacuolar lesion lesions are found consistently in the medulla oblongata (Fig. 3a), olfactory bulb, cortex, and hypothalamus. In elk, lesions are less severe in these locations. Hyperplasia and hypertrophy of astrocytes also may be observed. Amyloid plaques are common in deer brains stained with hematoxylin and eosin, but special staining of PrP by use of IHC analysis is necessary for demonstration of amyloid in elk brain. The PrPSc accumulation consistently in the parasympathetic dorsal motor nucleus of the vagus nerve (DMNV) (Fig. 3a).

Nor98 or atypical scrapie

An unusual presentation of scrapie in sheep was first recognized and described in Norway in 1998, and was designated Nor98. In this novel scrapie type, there were no indications of lateral or vertical transmission. Only single cases (1 sheep/flock) with a wide geographic distribution were observed, and it affected older animals of more resistant genotypes but not of the most susceptible genotypes. In clinical cases, ataxia, but not pruritus, was observed. Unlike typical scrapie, there was a preferential accumulation of PrPSc in the cerebellar cortex, with a peculiar IHC pattern not previously described, and there was absence or minimal PrPSc accumulation by use of IHC analysis of the medulla oblongata. The PrPSc was not detected in the LRS. Vacuolation, when present, affected mostly the cerebellum and cerebral cortex, but not the medulla oblongata.

Nor98 had a different from and characteristic glycoprofile of the WB analysis when a particular WB analysis (the TeSeE sheep/goat WB) was applied, showing a lower band of approximately 12 kD. It was postulated that Nor98 was a spontaneous form of TSE in animals. Following the Norwegian report, the unusual forms of scrapie were detected in several countries. Difficulties were encountered in the diagnosis of Nor98 in that some of the cases positive by use of the TeSeE ELISA were unconfirmed by histopathologic changes, and by results of IHC analysis and of several WB methods, including the WB analysis of the World Organization for Animal Health (OIE). However, when the TeSeE sheep/goat WB was applied to the obex and other brain areas, the peculiar Nor98-like profile was observed. Furthermore, if cerebellum was available, Nor98-like pathologic characteristics could be also confirmed by use of IHC analysis.

Atypical bovine spongiform encephalopathy

Two TSE cases with unusual features were identified in cattle in Italy and were named Bovine Amyloidotic Spongiform Encephalopathy (BASE). These cases differed from BSE in that they had amyloid plaques, predominantly in the white matter, that immunostained specifically for PrPSc. Plaques are rare in BSE, then are found mostly in the thalamus. Additionally, the distribution of PrPSc accumulation was different from that of BSE, with less involvement of the brain stem. The molecular signature also was dissimilar and resembled sCJD. Further indications that there could possibly be other forms of natural TSE disease in cattle originated in France. Three cases in old cattle had a different electrophoretic profile characterized by higher molecular mass of the unglycosylated PrPSc/band and high affinity for the MAb P4, compared with that for typical cattle BSE. Additionally, preliminary data from 2 BSE cases in young cattle in Japan indicated different biochemical characteristics. Several theories have been proposed to explain these atypical BSE presentations. One theory for Nor98 is that a spontaneous or sporadic form of TSE may occur in animals, like the sporadic form of human TSE-sCJD.

Diagnostic samples

Brain. Consistent early accumulation of PrPSc and vacuolar lesions in the medulla oblongata at the level of the obex, make this area of brain an optimal site for diagnosing BSE, CWD, and typical scrapie. For BSE, an appropriate sample for diagnosis should include the solitary and the trigeminal tract nuclei. For CWD and scrapie, it is critical that the DMNV is represented, particularly to detect early/asymptomatic infections. For atypical scrapie, the trigeminal tract nuclei and particularly the cerebellum are required for confirmation. Obex samples are conveniently obtained by introducing a commercially available, long, spoon-shaped metal or disposable instrument with cutting edges through the foramen magnum. In view of the emerging atypical cases in sheep, it is recommended that, besides the obex sample, cerebellum should also be submitted for TSE testing in small ruminants. Samples of cerebellum can also be taken through the foramen magnum after obtaining the
Figure 3. Photomicrographs of sections of nervous tissue from 2 wildlife species. a. Medulla oblongata (obex—parasympathetic dorsal motor nucleus of the vagus nerve) from an elk with chronic wasting disease (CWD). Immunohistochemical analysis (400×). b. Tonsil from a mule deer with CWD. Immunohistochemical analysis (250×).
obex sample by reintroducing the instrument, directed toward the cerebellum, rotating it, and drawing out a further sample. Levels of PrPSc in the brain are initially restricted to the aforementioned target areas, and may then increase and extend from these early target areas during the course of the disease. A large proportion of the TSE cases in the clinically healthy animals detected during surveillance at slaughter do not have histopathologic changes in the brain. This is due to the fact that PrPSc can be detected in the brain a few months before development of lesions and onset of clinical signs of disease.35,101

Unfixed (fresh) tissue for the screening test, and formalin-fixed tissue must be obtained for confirmatory and potentially discriminatory testing and/or strain characterization. The obex sample can be divided longitudinally, exactly along the midline, so that the right and left DMNV are not left on one of the samples. At some laboratories, the sample is divided in full transverse sections, one at the obex and another immediately adjacent, within 1.5 cm from the obex.63 If commercial sampling devices are used, it must be ensured that the target areas are actually picked. For active surveillance, the obex samples are obtained at slaughterhouses by trained personnel and are sent with proper identification, such as an ear-tag, to the testing laboratory. It is advisable to include a piece of the ear with the ear tag in case there is need to confirm by use of DNA analysis that the identity of the animal in the ear and the obex are in agreement. The importance of adequate and certain identification of the sample and of all records cannot be overstated. It is the experience of many laboratories that a positive BSE result is met with questioning about the identity of the sample and of the animal.

**Lymphoid tissues.** As described previously, PrPSc accumulates in lymphoid tissues in deer, elk, sheep, and goats. The consistency of this feature in deer and elk makes it the tissue of choice for diagnosis of CWD.39 In many scrapie cases, PrPSc is detected in the LRS earlier than in the brain, except for the sheep with genotypes with no peripheral PrPSc accumulation.42 Therefore, testing of brain and lymphoid tissue provides higher sensitivity of detection, at least in sheep42 and elk,85 but at this time, not in deer.39 Lymphoid tissues of choice, due to their accessibility for sampling and their close association with the gastrointestinal tract, are the medial retropharyngeal lymph nodes and the tonsils (Fig. 3b). Due to the consistent distribution of PrPSc in lymphoid follicles, it is important that the cortex of the lymph nodes is included in the sample.

**Autoysis.** The PrPSc is very resistant to degradation, and its detection is not significantly compromised by autoysis.18,22,99 Trials to evaluate the performance of rapid tests before approval by the EU now include a category of poor-quality samples, mostly autolytic.24 However, the early accumulation of PrPSc in the brain is very neuroanatomically restricted, and the biggest threat to sensitivity in the autolyzed tissues is the uncertainty as to whether the target areas were actually tested. In practice, most laboratories still need to test autolyzed material, often derived from fallen stock. Thus, the laboratory is responsible for recording and reporting that the sample was unsuitable and appropriately qualifying any negative result.

**Diagnostic methods: principles and application**

Historically, the initial diagnosis of TSE was based on histopathologic changes, but, given the pivotal role played by prion protein in pathogenesis, the characterization of PrPC and PrPSc has been a prime research element. Over time, many specific antibodies against PrP, the only unambiguous marker for TSE, have been produced. Immunology-based techniques, such as IHC3 and WB38 analyses, ELISA,20 and the conformation-dependent immunoassay (CDI),2,6 are being increasingly used to aid diagnosis for screening purposes, to study pathogenesis, and to provide comparative information at the molecular level. There are now many tests that have been validated and officially approved (Table 1), and several more are in the development stage. Most diagnostic methods for PrPSc detection rely on its protease resistance and identification by use of PrP-antibodies. However, a fundamental difficulty with antibodies to TSE is that those currently used cannot specifically discriminate PrPC from PrPSc.

In recent years, some MAbS have claimed to differentiate the two proteins.51,79,113 However, so far, none of these MAbS has proved suitable for direct identification of PrPSc by use of IHC analysis,2 and their application to other diagnostic tests remain to be seen. There is now a plethora of anti-PrP antibodies to various amino acid sequences of the PrP protein within the carboxyl, core, and N-terminal domains being produced. There are species differences in these sequences, whereby some antibodies raised against highly conserved sequences of the protein are very reactive in one species and poorly reactive in others. However, several factors are involved in achieving sensitive detection, such as the configuration of the protein as it unfolds and, therefore, the orientation and presentation of the epitope and the precise proteinase cleavage sites between different forms of the prion protein. These factors are generally controlled by the technique used, and it is often the case that antibodies that are good for IHC analysis are not necessarily suitable for WB analysis or ELISA, and vice-versa. Furthermore, the expression level of PrPC varies among tissue types, and as antibodies for nonneural tissue become more readily
available, they may not necessarily work well with brain.\textsuperscript{60}

\textbf{Antemortem diagnostic methods}

Currently, there are no reliable noninvasive tests for diagnosis of TSE in live animals or humans. Clinical signs of disease, although characteristic, are insufficient for a definitive diagnosis. It is assumed that the immune system fails to generate a specific response to PrP\textsubscript{Sc} because the protein is not recognized as “foreign.” Presently, immunologic tests that are based on detection of an immune response cannot be used for diagnosis of TSEs.

It has been shown that PrP\textsubscript{Sc} is present in blood and that TSE can be transmitted by blood transfusion.\textsuperscript{40} However, the PrP\textsubscript{Sc} concentration in blood is 100 to 1,000 times lower than that in the brain.\textsuperscript{35} The PrP\textsubscript{res} is also detected at low concentration in urine of human and animals affected by TSE.\textsuperscript{30} Even though the development of in vivo tests for urine or blood, (e.g., by immunocapillary electrophoresis) has made progress,\textsuperscript{77} sufficient sensitivity and consistency have not yet been achieved. Some tests already are commercially available, but their performance is not yet clearly established. Detection of PrP\textsubscript{Sc} in tissue biopsy specimens currently remains the only reliable method for diagnosing TSE in live individuals. Tonsillar biopsy specimens are used in cervids, small ruminants, and humans. In sheep, biopsy of the lymphoid deposits on the third eyelid also can be used,\textsuperscript{66} but in many cases, insufficient lymphoid tissue is obtained. The early distribution of PrP\textsubscript{Sc} in the lymphoid tissue is not homogeneous; therefore, it is considered that when applying IHC analysis, a minimal number (usually 4 to 6) of follicles with germinal centers needs to be examined to provide a reliable negative diagnosis. Brain biopsy is the last resort for confirmation of TSE in humans, and only a positive diagnosis is reliable.

\textbf{Rapid tests or screening tests}

These are tests of high sensitivity that can be completed within 24 hours, but usually are completed in a few hours, and are applicable to high throughput testing. For example, these may be used in slaughter house or hunter-killed populations. Many commercial rapid tests have been evaluated, and some are officially approved. Most of them are based on ELISA or WB methods, but IHC analysis also is used for screening for CWD (Table 1).

\textit{Enzyme-linked immunosorbent assays.} Several types of ELISAs have been developed for diagnosis of TSEs (Table 1) using brain (obex) or lymph node samples. The tissue is homogenized, subjected to PK treatment and centrifuged. The pellet is resuspended, diluted, and tested using an ELISA system.\textsuperscript{20,36} Various antibodies are used, and a list of companies supplying anti-PrP antibodies has been published.\textsuperscript{20} The PrP\textsubscript{res} is captured on a microtiteration plate, using anti-PrP antibody, or alternatively, a nonbiological ligand. A second anti-PrP antibody, coupled to various detection systems, is then used for detection of PrP\textsubscript{res}. The originally described CDI relied on the detection of conformational differences of PrP isoforms among experimental scrapie strains, without preliminary protease digestion, by quantifying the relative binding of antibodies to denatured and native protein.\textsuperscript{76} This test\textsuperscript{4} has now been modified and developed into a commercial ELISA for detection of abnormal PrP in cattle.

\textit{Western immunoblot analysis.} Initially, WB protocols were lengthy procedures, with several ultracentrifugation steps needed to purify the PrP\textsubscript{Sc}.\textsuperscript{91} The rapid WB tests have been adapted to omit the ultracentrifugation steps by the use of appropriate homogenization buffers. Following usual electrophoretic separation on polyacrylamide gels and transfer onto membranes, the abnormal PrP protein is detected after incubation with an antibody against the protease-resistant core of the protein, then with a conjugated secondary antibody. Detection of the PrP\textsubscript{res} signals generally involves use of a chemiluminescent substrate.\textsuperscript{88}

\textbf{Confirmatory tests}

Tests of high specificity are used to confirm clinical suspicion of disease or positive or inconclusive rapid test results. In most countries, only confirmed BSE and scrapie cases are considered positive. The methods that are recommended as confirmatory tests have been described and consist of histologic examination, IHC analysis, detection of scrapie-associated fibril (SAF), and WB analysis.\textsuperscript{63,64}

\textit{Histologic examination.} Positive diagnosis of BSE, scrapie, or CWD can be based on histopathologic changes alone when the characteristic vacuolar changes in the brain, with the typical neuroanatomic distribution, are recognized.\textsuperscript{83,100,105} Other histologic features of TSE, such as presence of florid plaques, astrogliosis, and neuronal loss, when present, support a positive diagnosis, but are not diagnostic per se in the absence of TSE vacuolation. Diagnosis in cases with no lesions and mild or equivocal changes needs to be confirmed by use of more sensitive methods, such as IHC or WB analysis.

\textit{Immunohistochemical analysis.} Recognizing the presence of disease-specific immunostaining in the target areas and with the characteristic patterns is the most specific test for TSE available so far. Immunohistochemical analysis usually is conducted on formalin-fixed samples. It involves use of pretreatment of the sections with formic acid and hydrated autoclaving for epitope demasking\textsuperscript{18} and to remove the normal
<table>
<thead>
<tr>
<th>Name of test</th>
<th>Principle†</th>
<th>Approved‡</th>
<th>Comments§</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rapid tests/screening tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-Rad TeSeE®, (former Platelia test®)</td>
<td>Sandwich ELISA. Uses PK treatment, 2 MAbs and one concentration step</td>
<td>EU approved for cattle in 2001 and small ruminants²⁶</td>
<td>Used on large scale in Europe and USA. Good discrimination between positives and negatives. Full automation is possible. Good detection of Nor98. BSE (cattle) Sn: 100 (CI:99), Sp:100 (CI 99.7)</td>
</tr>
<tr>
<td>Enfer testf</td>
<td>Chemiluminescent simple ELISA. Detect PK-resistant PrPSc using a polyclonal antibody</td>
<td>EU approved for cattle in 2001 and small ruminants²⁶</td>
<td>Fewer than 4 hours to complete. BSE (cattle) Sn: 100 (CI:99.5), Sp: 100 (CI 99.7)</td>
</tr>
<tr>
<td>Enfer TSE kit version 2.0g</td>
<td>Same as above, but with automated sample preparation</td>
<td>EU approved for cattle 2005²⁶</td>
<td>For BSE (cattle) Sn: 100 (CI 98.5), Sp: 100 (CI 99.97). Sn in poor-quality samples: 100 (CI 98.5)²⁴</td>
</tr>
<tr>
<td>Prionics-Check WESTERN testa</td>
<td>WB, uses MAb 6H4 as primary antibody</td>
<td>EU approved for cattle 2001 and small ruminants²⁶</td>
<td>Not a quantitative test, not automated, 6–8 hours to complete. For CWD in mule and WT deer in RLN: Sn: 100.0 (CI 97.4), Sp: 100.0 (CI 99.8); in obex: Sp: 100.0 (CI 99.5). CWD in RM elk in RLN: Sn: 93.8 (CI 65.2–99.8), Sp: 100.0 (CI 97.4)²³ BSE (cattle) Sn: 100 (CI 99.9), Sp:100 (CI 99.7)</td>
</tr>
<tr>
<td>Prionics-Check LIA (Prionics/Roche)³</td>
<td>Luminescence sandwich ELISA</td>
<td>EU approved for cattle in 2003 and small ruminants²⁶</td>
<td>No centrifugation stage. For CWD in mule and WT deer in RLN: Sn: 100.0 (CI 98.4), Sp: 99.7 (CI 98.9); in obex: Sn 100 (CI 92.5), Sp: 99.8 (CI 99.2). CWD in RM elk in RLN: Sn: 84.1 (CI: 67.2–93.6), Sp: 99.0 (97.5), in obex: Sn: 100.0 (CI 70.0), Sp: 100.0 (CI 99.5)²³</td>
</tr>
<tr>
<td>InPro CDI-5 (conformational dependent immunooassay)⁴</td>
<td>Sandwich ELISA. Detects all PrP in sample. Comparison of the native (PrPSc) with total (PrPpos and PrPf) informs on the level of abnormal PrP in the sample</td>
<td>EU approved for cattle in 2003 and small ruminants²⁶</td>
<td></td>
</tr>
<tr>
<td>CediTect BSE test⁶</td>
<td>PVDF filter chemiluminescence ELISA for detection of PrPpos using an MAb</td>
<td>EU approved for cattle 2005²⁶</td>
<td>For BSE (cattle): Sn: 99.5 (CI 97.7), Sp: 100 (CI 99.97). Sn in poor-quality samples: 100 (CI 98.6)²³</td>
</tr>
<tr>
<td>IDEXX HerdChek BSE antigen test kit (EIA)⁷</td>
<td>Antigen capture ELISA, uses one MAb, no PK treatment</td>
<td>EU approved for cattle, 2005²⁶</td>
<td>For CWD, mule and WT deer, in RLN: Sn: 96.8 (CI 94.4–98.4), Sp: 99.8 (CI 99.6). For BSE (cattle): Sn: 100 (CI 98.5), Sp: 99.99 (CI 99.95). Sn in poor quality samples: 100 (CI 98.5)²³ For BSE (cattle): Sn: 100 (CI 98.5), Sp: 100 (CI 99.97). Sn in poor-quality samples: 100 (CI 98.5)²³</td>
</tr>
<tr>
<td>Institut Pourquier Speed’it BSE⁵</td>
<td>Chemiluminescence ELISA, detects PrPpos using an MAb</td>
<td>EU approved for cattle 2005²⁶</td>
<td>For BSE (cattle): Sn: 100 (CI 98.5), Sp: 100 (CI 99.97). Sn in poor-quality samples: 100 (CI 98.5)²³</td>
</tr>
<tr>
<td>Prionics Check PriO-STRIP®</td>
<td>Lateral flow immunoassay with two different MAbs to detect PrPpos</td>
<td>EU approved for cattle 2005²⁶</td>
<td>For BSE (cattle): Sn: 100 (CI 98.5), Sp: 100 (CI 99.97). Sn in poor-quality samples: 99.6 (CI 97.9)²³</td>
</tr>
<tr>
<td>Roboscreen Beta Prion BSE EIA test kit⁶</td>
<td>Uses two MAbs against two epitopes in a highly unfolded state of bovine PrPpos in a two-sided immunoassay</td>
<td>EU approved for cattle 2005²⁶</td>
<td>For BSE (cattle): Sn: 100 (CI 98.5), Sp: 100 (CI 99.97). Sn in poor quality samples: 100 (CI 98.5)²³</td>
</tr>
</tbody>
</table>
Table 1. Continued.

<table>
<thead>
<tr>
<th>Name of test</th>
<th>Principle</th>
<th>Approved</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche Applied Science PrionScreen&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Sandwich ELISA for detection of PrP&lt;sub&gt;Sc&lt;/sub&gt; in streptavidin-coated microplates after PK digestion</td>
<td>EU approved for cattle 2005&lt;sup&gt;26&lt;/sup&gt;</td>
<td>For BSE (cattle): Sn: 100 (CI 98.5), Sp: 100 (CI 99.97). Sn in poor quality samples: 10 (CI 98.5)&lt;sup&gt;24&lt;/sup&gt; Not marketed anymore</td>
</tr>
<tr>
<td>VMRD CWD dbELISA&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Dot blot ELISA on 96 well plates using one MAb</td>
<td>USDA licensed in 2002 for RLN of mule and WT deer</td>
<td>Good detection of TSE in small ruminants, including Nor98. Results in 5 hours. For CWD in mule and WT deer, in RLN: Sn: 100.0 (CI 98.8), Sp: 100.0 (CI: 99.7), in obex: 96.0 (CI 81.0), Sp: 85.7 (CI 20.4–99.6). CWD in RM elk, in RLN: Sn: 97.3 (CI 84.9–99.9), Sp: 100.0 (CI 98.5); in obex: Sn: 88.9 (CI: 59.9–98.7), Sp: 100.0 (CI: 57.2)&lt;sup&gt;23&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bio-Rad TeSeE sheep/goat</td>
<td>ELISA adapted to sheep and goat testing</td>
<td>Awaiting for official EU approval</td>
<td></td>
</tr>
<tr>
<td>BioRad CWD ELISA&lt;sup&gt;−39&lt;/sup&gt;</td>
<td>Sandwich ELISA</td>
<td>USDA licensed in 2002 for RLN in wild mule deer, WT deer, and elk</td>
<td></td>
</tr>
<tr>
<td>Bio-Rad TeSeE test kit</td>
<td>Modified BioRad CWD Sandwich ELISA to allow automation of sample preparation</td>
<td>Second-generation test kit. USDA licensed in 2003 for wild mule deer, WT deer, and elk</td>
<td>Automated robotic testing platform speeds up processing to allow testing up to 1,000 samples/d. For CWD in mule and WT deer, in RLN: Sn: 100.0 (CI 98.1), Sp: 100.0 (CI 99.7). CWD in RM elk, in RLN: Sn: 100.0 (CI 75.0), Sp: 100.0 (CI 98.5)&lt;sup&gt;23&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ventana IHC Systems&lt;sup&gt;1&lt;/sup&gt; and VMRD Prion Protein Detection Kit&lt;sup&gt;1,86&lt;/sup&gt;</td>
<td>Automated IHC staining of PrP&lt;sub&gt;CWD&lt;/sub&gt; in sections of brain or lymphoid tissue with MAb F99.98/1.6&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Official APHIS test for CWD surveillance in captive cervids</td>
<td>Used as screening or confirmatory test. Three to 5 days to complete, depending on fixation time Results in 3.5 hours</td>
</tr>
<tr>
<td>IDEXX HerdChek CWD Antigen EIA Test&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Antigen-capture ELISA, uses one MAb, no PK treatment</td>
<td>USDA licensed in 2003 for RLN of mule and WT deer</td>
<td></td>
</tr>
<tr>
<td>PDL CWD Rapid Antigen Test&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Lateral flow strip test</td>
<td>USDA licensed in 2004 for RLN of WT deer</td>
<td>Simple, no special equipment required</td>
</tr>
<tr>
<td>Confirmatory tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLA Discriminatory WB technique&lt;sup&gt;a&lt;/sup&gt;</td>
<td>VLA Modified Prionics-Check Western test&lt;sup&gt;1&lt;/sup&gt;. An added centrifugation step aids computer analysis uses MAb 6H4&lt;sup&gt;a&lt;/sup&gt; for cattle and MAb P4&lt;sup&gt;b&lt;/sup&gt; for sheep</td>
<td>Prionics component. EU approved in 2001 for cattle and small ruminants&lt;sup&gt;26&lt;/sup&gt; Recommended by OIE&lt;sup&gt;80,64&lt;/sup&gt;</td>
<td>Used as statutory test for BSE and scrapie in the UK. Also used as discriminatory test Requires large starting weight (4 g) of material. Laborious and lengthy procedures. Currently less used, replaced by other WB methods Used as screening or confirmatory test. Three to 5 days to complete, depending on fixation time Most specific test for TSE. Three to 5 days to complete, depending on fixation time. Target areas for PrP&lt;sub&gt;Sc&lt;/sub&gt; accumulation must be represented in the section(s). Sensitivity affected by protocol used (proficiency testing of approved laboratories is recommended) Cases may be detected as part of routine diagnostic histologic examination. Mostly used together with IHC analysis if suspicion of TSE. Lower sensitivity than IHC, WB, and rapid tests Low sensitivity, suitable for autolyzed and/or formalin-fixed tissues</td>
</tr>
<tr>
<td>OIE WB technique&lt;sup&gt;63,64&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventana IHC Systems&lt;sup&gt;1&lt;/sup&gt; and VMRD Prion Protein Detection Kit&lt;sup&gt;1,86&lt;/sup&gt; Immunohistochemical analysis&lt;sup&gt;63&lt;/sup&gt;</td>
<td>Automated IHC staining of PrP&lt;sub&gt;CWD&lt;/sub&gt; in sections of brain or lymphoid tissue with MAb F99.98/1.6&lt;sup&gt;1&lt;/sup&gt; Many protocols and antibodies used in various laboratories</td>
<td>Official APHIS test for CWD surveillance in captive cervids Recommended by OIE&lt;sup&gt;80,64&lt;/sup&gt; The use at NRL is approved by CRL after proficiency testing of the results of the IHC technique and interpretation</td>
<td></td>
</tr>
<tr>
<td>Histologic examination&lt;sup&gt;63&lt;/sup&gt;</td>
<td>Recognition of pathognomonic lesions in HE-stained brain sections</td>
<td>Recommended by OIE&lt;sup&gt;80,64&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Scrapie-associated fibrils (SAF)</td>
<td>Detection of purified scrapie-associated fibrils by negative-contrast electron microscopy&lt;sup&gt;17,18,91,92&lt;/sup&gt;</td>
<td>Recommended by OIE&lt;sup&gt;80,64&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>
PrP\(^c\) (see CRL website as previously cited).\(^{98}\) Some protocols also include proteolytic digestion. A wide range of anti-PrP monoclonal and polyclonal antibodies can be used, and an increasing number are now commercially available. Some perform better on a particular species, and others are, in fact, species specific. Immunohistochemical analysis also is used as a screening test for CWD in automated high output systems, and it is used as an official test for CWD surveillance in captive cervids in the USA (http://www.aphis.usda.gov/lpa/issues/cwd/cwdposition.html).

Several immunohistochemical methods have been described for TSE.\(^{63,86}\) When working with BSE material, many laboratories include concentrated formic acid treatment of the tissue blocks to decrease the infectivity levels. It also is a recommended practice to dip the final coverslipped slides in formic acid before they are dispatched from the containment facilities.\(^{93}\)

**Scrapie-associated fibrils.** In the early days, histologic examination and detection of SAF by use of negative-contrast electron microscopy were the only available tests for diagnosis of TSE.\(^{91,92}\) Today, SAF is rarely used because of its comparatively low sensitivity, but it may be a method of choice when only severely autolysed\(^{98}\) or only formalin-fixed material is available.\(^{57}\)

**Western immunoblot analysis.** The first WB technique to be used for diagnostic purposes was the OIE confirmatory WB,\(^{88,91}\) which used a relatively large amount of starting material (4 g), a detergent extraction, lengthy ultracentrifugation steps to concentrate any PrPres\(^c\) and PK digestion to eliminate PrP\(^c\). Current WB techniques use smaller quantities of brain material, (up to 0.5 g), and due to the use of appropriate combinations of homogenization buffers and antibodies, do not need the ultracentrifugation steps. With use of highly sensitive antibodies, most have an analytical sensitivity comparable to that of the most sensitive ELISA-based rapid tests. Precipitation of PrP\(^\text{Sc}\) by use of reagents such as sodium phosphotungstic acid increase test sensitivity and allows detection of PrPres\(^c\) in non-neural tissues, and has also been applied to a WB technique.\(^{88}\)

### Strain discriminatory and typing methods

Most methods used to identify/characterize prion strains have been developed through the urgent need to differentiate BSE from scrapie in sheep. The original definition of a TSE strain was: an agent that induces constant features of spongiform lesions and incubation periods when serially passed through congenic mice. However, this definition does not cover recent scientific discoveries, as more and more data on strain differences accumulate. These include molecular tests, results of atypical cases, IHC differentiation at the cellular level, and use of transgenic mice. In vitro methods used to give a preliminary molecular description of the isolate cannot actually identify a strain. The ultimate typing of the prion strain is still determined by

---

### Table 1. Continued.

<table>
<thead>
<tr>
<th>Name of test</th>
<th>Principle†</th>
<th>Approved‡</th>
<th>Comments§</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Discriminatory tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLA Discriminatory WB technique(^a)</td>
<td>VLA Modified Prionics-Check</td>
<td>Evaluated and approved</td>
<td>MAb P4 selective for scrapie, but</td>
</tr>
<tr>
<td></td>
<td>Western test(^a) adding detection with MAb P4(^a)</td>
<td>as discriminatory test by CRL in 2005</td>
<td>not BSE under these test conditions. Used also as confirmatory test</td>
</tr>
<tr>
<td>AFSSA Discriminatory WB(^b)</td>
<td>Uses MAbS BAR233(^a) and P4(^b)</td>
<td>Evaluated and approved</td>
<td>See example in Fig. 4.</td>
</tr>
<tr>
<td></td>
<td>Shows molecular differences between scrapie and BSE that result from different resistance to PK digestion, and differential immunoreactivity</td>
<td>as discriminatory test by CRL in 2005</td>
<td></td>
</tr>
<tr>
<td>DTST (Discriminatory test for strain typing of TSE in small ruminants), CEA method(^d)</td>
<td>WB method based on different resistance to proteolysis of the N-terminal regions of PrP(^\text{Sc}) depending on TSE strain. Uses a modified TeSeE sheep/goat WB(^d) with MAb Saf-37</td>
<td>Evaluated and approved as discriminatory test by CRL in 2005</td>
<td>Can also differentiate BSE and scrapie from strains with higher PK sensitivity, such as Nor98</td>
</tr>
</tbody>
</table>

* Superscripted numerals are reference citations.
† PK = protease K; MAbS = monoclonal antibodies; IHC = immunohistochemical (analysis); HE = hematoxylin and eosin.
‡ EU = European Union; USDA = United States Department of Agriculture; RLH = retropharyngeal lymph node; APHIS = Animal and Plant Health Inspection Service (USA); CRL = EU Community Reference Laboratory; OIE = world Organization for Animal Health; NRL = National Reference Laboratories (Europe).
§ LIA = luminescent immunoassay; PVDF = [sn9]; Sn = sensitivity in %; CI = confidence interval (95%), %, Sp = specificity in %, RM = Rocky Mountain elk; WT deer = white-tailed deer.
use of bioassay, as molecular characterizations are not fully specific.

In vitro methods for preliminary strain discrimination. These methods are used as an initial screen to characterize the molecular properties of abnormal PrP and its possible similarities to the BSE agent. The use of screening tests allows rapid application of control measures to the flock of origin of the BSE suspect strain prior to further investigations. Five methods, including 3 WB protocols, an ELISA, and an IHC protocol, were initially validated by examination of samples of BSE in experimentally infected sheep and scrapie. All of these methods are based on the particular higher sensitivity of the BSE agent to PK digestion. The precise basis for discrimination is the location of the N-terminal cleavage site for PK digestion of PrP between BSE and scrapie. It has been shown, by use of antibody-blocking techniques and Pepscan analysis, that the N-terminal amino acid sequence WGQGGSH remained intact only in sheep scrapie samples, thus being detected by an antibody specific to this sequence in the N-terminal domain, in this case MAb P4. Figure 4 and Table 2 illustrate the application of one EU-approved Western blot protocol for preliminary discrimination. Some of the WB tests have the ability to detect atypical scrapie and atypical BSE cases, and some are able to discriminate between BSE, natural and experimental scrapie cases, and experimental BSE in sheep cases. A recent study, albeit on a small number of samples, indicated that it was also possible to discriminate between CWD in elk and white-tailed deer, and BSE and scrapie cases.

Differential IHC analysis. Initially, it had been shown that scrapie and experimental BSE in sheep could be distinguished by the proportion of pathogenic PrP accumulation relative to that in various cell types in the brain, and by different labeling patterns of PrP peptide sequences in phagocytic cells. Differential IHC methods were further developed applying various peptide-specific antibodies directed against the PK cleavage region of the PrP. A more extensive digestion of the abnormal PrP was shown in neurons, glial cells, and TBM of the LRS in BSE-infected sheep, compared with that in scrapie-infected animals. With preclinical BSE, single granules were observed in TBM in the ovine tonsils, but clusters were seen in scrapie. Bioassays. Bioassays, the experimentally induced infection of animals, have several applications in diagnostics and research, and they are used as the definitive strain-typing method. They also provide the ultimate documentation of PrPSc infectivity, either to confirm a TSE case or in end-point titration studies to estimate the degree of prion infectivity of various materials and tissues, such as those used in pathogenesis studies. Bioassays have the highest sensitivity when conducted in homologous species (e.g., for BSE, the calf bioassay is more sensitive than is the mouse bioassay). Bioassay in mice conducted with brain material from humans, cats, or exotic ungulates infected with BSE revealed one unique and similar incubation pattern and lesion profile. This indicated that the infection in all of these hosts had been caused by a single BSE strain with stable biological properties. On the other hand, many TSE strains have been detected in small ruminants. Bioassays are lengthy (the mean incubation period for BSE in RIII mice is 408 days) and labor intensive, and results are not always easy to interpret. Additionally, there is a species barrier to infection, which means that not all isolates will necessarily infect an experimental host. These restrictions have led to a burgeoning number of transgenic (Tg) mice carrying human, sheep, deer, elk, or cattle PrP genes. These models have the potential to increase sensitivity by their genetic closeness to a specific natural host, and to reduce duration of the incubation period. However, there is a growing tendency for Tg mouse models to be used for applications that have not been comprehensively assessed.

Lesion profiling. Scoring the intensity of neuropil vacuolation in a selected set of neuroanatomic locations and representing the scores versus location in a linear graph, results in a curve called the TSE profile. The severity of lesions may vary among animals, reflecting the stage in the course of the disease; advanced cases have the most severe vacuolation. However, the shape of the curve, remains consistent for a particular agent/host combination. Lesion profiling is conducted on the definitive hosts (e.g., cattle, sheep, goats), or on experimentally infected animals, such as mice, for the application described previously. Profiling for BSE in cattle indicated that the pathologic profile was stable, implying the presence of a single strain or strains indistinguishable by use of these parameters in various breeds and during the course of the epidemic. For CWD, the distribution of lesions in mule deer has been well characterized, but profiling curves in the natural cervid hosts or in laboratory animals have, to our knowledge, not been published.

New developments

A substantial amount of research and development aims at producing diagnostic tests with higher sensitivity by increasing PrPSc detection, by concentrating or by amplifying the PrPSc. More sensitive tests could be applied with higher reliability to: nonneural tissues; clinical samples, such as blood or urine; samples obtained at an earlier stage in the incubation period; or pooled samples from several animals using smaller amounts of material. An example of a new approach...
**WB with Mab Bar233**

<table>
<thead>
<tr>
<th>MW</th>
<th>Scapie case</th>
<th>Control</th>
<th>BSE in sheep</th>
<th>Undetermined</th>
<th>BSE in sheep</th>
<th>BSE in sheep</th>
<th>MW</th>
<th>Undetermined</th>
<th>BSE in sheep</th>
<th>Control</th>
<th>BSE in sheep</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.255</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>++</td>
<td>-</td>
<td>++</td>
<td></td>
<td></td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-0.428</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-0.653</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>++</td>
<td>-</td>
<td>++</td>
<td></td>
<td></td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-0.357</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**WB with Mab P4**

<table>
<thead>
<tr>
<th>MW</th>
<th>Scapie case</th>
<th>Control</th>
<th>BSE in sheep</th>
<th>Undetermined</th>
<th>BSE in sheep</th>
<th>BSE in sheep</th>
<th>MW</th>
<th>Undetermined</th>
<th>BSE in sheep</th>
<th>Control</th>
<th>BSE in sheep</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.255</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>++</td>
<td>-</td>
<td>++</td>
<td></td>
<td></td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-0.428</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-0.653</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>++</td>
<td>-</td>
<td>++</td>
<td></td>
<td></td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-0.357</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Discriminatory Western Immunoblotting is applied to provide a preliminary discrimination between scrapie and possible BSE ("suspect") in sheep. Two criteria are used for the molecular characterization of transmissible spongiform encephalopathy (TSE) in sheep: the difference of molecular weight (Dmw) and of immunoreactivity. The Dmw consists of the difference between the molecular weight of the unglycosylated band of the test sample (2, 4, 5, 7, 9, 11, 12, 14) and the molecular weight of the unglycosylated band of control (Cattle BSE) (3, 6, 10, 13). The difference in immunoreactivity is the difference between signals with monoclonal antibodies (MAbs) P4 and Bar 233. The sample (2) with a positive Dmw, compared with that of cattle BSE samples (3) behaves like most natural scrapie cases; this case is also strongly labeled by P4 MAb. In contrast, samples (4, 7, 9, 12, 14) with a lower Dmw, compared with that of cattle BSE samples (3, 6, 10, 13), and only faint labeling with MAb P4 behave like BSE in sheep and should be considered as BSE suspects. Samples with low levels of PrPc (5, 11) remain undetermined (Und). MW = molecular weight control. MAb Bar 233 and MAb P4 recognize the 145–156 and 89–104 regions of the ovine PrP protein, respectively. Dmw = MW unglycosylated band of test sample – MW unglycosylated band of control BSE sample. The cut-off for the Dmw is 0.100. Table 2 aids the interpretation of the discriminatory Western blotting in Figure 4.

is the protein misfolding cyclic amplification, which allows in vitro conversion of PrPc into PrPsc in the presence of PrPc as substrate and of minute amounts (undetectable levels) of PrPsc template. The PrPsc aggregates formed are disrupted by sonication to produce smaller units of PrPsc for continued formation of new PrPsc. Several prion-susceptible cell lines have been developed and are used in research to study the bio-
logical mechanisms of PrPSc propagation. This in vitro method could provide a replacement for bioassay, at least for prion infectivity end-point titrations.9

General discussion

The choice of diagnostic methods depends on the purpose of the testing: screening, confirming TSE, or typing a strain. It was clearly stated by the USDA that the current available tests for CWD could only be applied as a surveillance tool and not for food safety purposes. Likewise, the EU has approved tests solely for surveillance. The reasons why current tests are not considered to be appropriate for food safety control are that there is not sufficient knowledge about: the infective dose for development of natural TSE in animals and humans, the PrPSc tissue distribution and infectivity, and the stage in the incubation period at which the current diagnostic tests are able to detect natural infection.

With the arsenal of diagnostic tests for TSE available today, the postmortem diagnosis of well established infection poses no major difficulties. On the other hand, tests applied to early-stage infection, in which the accumulation of PrPSc is minimal or at just about detection limits of the test may yield false-negative or inconclusive results. Screening tests of high sensitivity (e.g., TeSeE ELISA) may either detect such cases as positive or may classify them as “suspect,” and confirmation of results may be more difficult. This was exemplified by the recent BSE case in the USA, in which the application of 2 confirmatory tests was necessary for corroboration.1 Discrepancies in results may also derive from differences in protocols at different laboratories, particularly for in–house-developed IHC and WB methods. More data are available on the performance of commercial rapid tests, several of which have been validated for specific applications and are officially approved (Table 1). For example, the European Food Safety Authority (EFSA) organized a working group that evaluated data provided by companies producing tests for diagnosis of CWD (http://www.efsa.eu.int/science/biohaz/biohaz_opinions/opinion_annexes/500_en.html), and concluded that all the tests evaluated would be suitable for surveillance in Europe. The information about sensitivity and specificity of the various tests may derive from data obtained by the companies producing the tests or by independent validation studies, which may have been conducted using different designs on different numbers of tested animals, or using different reference tests and material. For example, IHC analysis was used as the “gold standard” for a validation study for the Bio-Rad ELISA for CWD,19 whereas 2 other reference tests, the Bio-Rad TeSeE and the Prionics Check Western were used by the EFSA for the before-approval evaluation of 7 new rapid BSE tests.24 Therefore, direct comparisons of sensitivity and specificity of the tests are often not appropriate.

Future perspectives

It is difficult to predict with any certainty the future ground-breaking events that will occur with this most enigmatic group of diseases. At times, it seems as though no progress has been made since the confirmatory diagnosis is still made after death of the subject. As new tests are generated, more problems seem to appear, such as the appearance of atypical scrapie cases in the less susceptible genotypes, and possible different phenotypes of BSE. However, these are probably cases that previously were undetectable by use of the less sensitive tests of the past. In the future, there is likely to be a greater emphasis on preclinical disease status, expanding use of Tg mice with shorter incubation periods, more frequent use of antemortem biopsy for early diagnosis, and ultimately, the ideal for TSE diagnosis (i.e., an ultrasensitive antemortem test.

Table 2. Aid for the interpretation of the discriminatory Western blotting in Fig. 4.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Immunoreactivity</th>
<th>Dmw</th>
<th>P4</th>
<th>Bar 233</th>
<th>Other</th>
<th>TSE status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immunoreactivity</td>
<td></td>
<td>P4</td>
<td>Bar 233</td>
<td></td>
<td>Scrapie</td>
</tr>
<tr>
<td>(lane 2)</td>
<td>Dmw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Scrapie</td>
</tr>
<tr>
<td>Sample 2</td>
<td>Dmw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Scrapie</td>
</tr>
<tr>
<td>(lane 4)</td>
<td>Immunoreactivity</td>
<td></td>
<td>P4</td>
<td>Bar 233</td>
<td></td>
<td>Suspect</td>
</tr>
<tr>
<td>Sample 3</td>
<td>Dmw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Suspect</td>
</tr>
<tr>
<td>(lane 5)</td>
<td>Immunoreactivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Should be repeated</td>
</tr>
<tr>
<td>Sample 4</td>
<td>Dmw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Suspect</td>
</tr>
<tr>
<td>(lane 7)</td>
<td>Immunoreactivity</td>
<td></td>
<td>P4</td>
<td>Bar 233</td>
<td></td>
<td>Suspect</td>
</tr>
<tr>
<td>Sample 5</td>
<td>Dmw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Suspect</td>
</tr>
<tr>
<td>(lane 9)</td>
<td>Immunoreactivity</td>
<td></td>
<td>P4</td>
<td>Bar 233</td>
<td></td>
<td>Suspect</td>
</tr>
<tr>
<td>Sample 6</td>
<td>Dmw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Should be repeated</td>
</tr>
<tr>
<td>(lane 11)</td>
<td>Immunoreactivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Suspect</td>
</tr>
<tr>
<td>Sample 7</td>
<td>Dmw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Suspect</td>
</tr>
<tr>
<td>(lane 12)</td>
<td>Immunoreactivity</td>
<td></td>
<td>P4</td>
<td>Bar 233</td>
<td></td>
<td>Suspect</td>
</tr>
<tr>
<td>Sample 8</td>
<td>Dmw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Suspect</td>
</tr>
<tr>
<td>(lane 14)</td>
<td>Immunoreactivity</td>
<td></td>
<td>P4</td>
<td>Bar 233</td>
<td></td>
<td>Suspect</td>
</tr>
</tbody>
</table>
capable or rendering a diagnosis early in the course of the disease).

Acknowledgements

The authors are grateful to Yvonne Spencer, Paul Webb, Melanie Chaplin, and John Spiropoulos. The authors especially thank Gerald Wells for the scrapie and BSE photographs, Johann Vulin for the WB photograph, Danny Matthews for useful advice, and Bengt Ekberg for technical help with the illustrations.

Sources and manufacturers

a. Roche Diagnostics GmbH, Mannheim, Germany.

b. RIDA, R-Biopharm Rhone LTD, Glasgow, Scotland.
d. InPro, San Francisco, CA.
e. AFSSA, Lyon, France.
g. CEDI Diagnostics, Lelystad, The Netherlands.
h. IDEXX Laboratories Inc., Westbrook ME.
i. Institut Pourquier, Montpellier, France.
j. Prionics AG, Schlieren, Switzerland.
k. Roboscreen GmbH, Leipzig, Germany.
l. VMRD Inc., Pullman WA.
m. Ventana IHC Systems, Tucson, AZ.

n. Prion Development Laboratories Inc., Efoora, Buffalo Grove, IL.
o. Veterinary Laboratory Agency, Weybridge, UK.
p. Supplied by J. Grassi, CEA Saclay, France.
q. CEA, Fontenay-aux-Roses, France.

References


29. Fraser JR: 2002, What is the basis of transmissible spongiform...
encephalopathy induced neurodegeneration and can it be repaired? Neuropathol Appl Neurobiol 28:1–11.


88. Stack MJ, Balachandran A, Chaplin M, Davis L: 2004, The first Canadian indigenous case of bovine spongiform encephalopathy (BSE) has molecular characteristics for prion protein that are similar to those of BSE in the United Kingdom but differ from those of chronic wasting disease in captive elk and deer. Can Vet J 45:825–830.


