CHAPTER 2.7.13.

SCRAPIE

SUMMARY

Scrapie is a naturally occurring, infectious, neurodegenerative disease of sheep and goats characterised by vacuolar or spongy changes in the central nervous system (CNS). It has been recognised as a clinical disorder for more than 250 years, and is now classified as a transmissible spongiform encephalopathy (TSE), or prion disease, defined by the accumulation of an abnormal form (referred to as PrPSc) of a host membrane glycoprotein (prion protein or PrP), in the CNS. In some animals, PrPSc accumulation is also detectable in lymphoreticular tissues. Polymorphisms of the sheep PrP gene are associated with susceptibility to scrapie. PrP genotyping has been used as a tool in the control of classical scrapie, but no genotype appears to be completely resistant to infection, and the recently identified variant – atypical scrapie – has been reported in sheep of PrP genotypes that are apparently resistant to classical scrapie. Classical scrapie is endemic in many parts of the world, where it has often been introduced by importation. Australia and New Zealand have maintained freedom by use of strict restrictions on imports and other measures. The infection in sheep may be passed from ewe to lamb in the period from parturition to weaning. Infection can also pass horizontally to unrelated sheep or goats, especially when parturition occurs in confined areas. Fetal membranes are known to be a source of infection, and sheep milk from clinically affected animals has been demonstrated to transmit disease. The incubation time between primary infection and clinical disease is nearly always longer than 1 year and may sometimes exceed the commercial lifespan of the sheep. The majority of cases occur in sheep between 2 and 5 years of age. Clinical disease only develops if the infection enters the CNS. Atypical scrapie, when it presents clinically, appears to affect older animals predominantly, and occurs with a geographical distribution suggestive of a spontaneous disease, although it can be transmitted experimentally.

Identification of the agent: The disease is recognised by the clinical signs, which are variable, but generally start insidiously with behavioural abnormalities then progress to more obvious neurological signs, including pruritus, incoordination and poor bodily condition. Clinical diagnosis is confirmed by the presence of gray matter vacuolation of target areas within the brain tissue and/or the immuno-detection of disease-specific accumulations of PrPSc in the brain or lymphoreticular tissues. Immunochemical detection of the protein in brain samples forms the basis of rapid tests used in active surveillance programmes. In experimental studies of the disease in sheep and goats, detectable PrPSc accumulation in the brain does not start until several months after challenge, so it must be remembered that a negative result does not necessarily equate to an unexposed animal.

Detection of PrPSc in lymphoreticular tissues during the incubation period of scrapie in some animals offers a means of preclinical diagnosis of scrapie infection and may be particularly useful for surveillance purposes. It can also be performed in vivo using biopsied tissue. It is not, however, appropriate for atypical cases, or a proportion of classical cases, so can only be used to confirm presence of infection and cannot be used to prove absence of disease.

Most, but not all, currently recognised forms of scrapie can be transmitted to laboratory rodents by injecting them with infected brain tissue, but the variable efficiency of transmission coupled with long incubation times preclude this as a practical diagnostic procedure.

Serological tests: Scrapie infection is not known to elicit any specific immune response and there is no basis for establishing a diagnosis by detecting specific antibodies.

Requirements for vaccines and diagnostic biologicals: There are no biological products available.
A. INTRODUCTION

Scrapie (also known as la tremblante; Traberkrankheit; Gnubberkrankheit) is a naturally occurring progressive, fatal, infectious, neurodegenerative disease of sheep and goats that has been recognised for at least 250 years, and has been reported in Europe, North America, Asia and Africa. Scrapie and other related diseases in humans and animals were later classified as transmissible spongiform encephalopathies (TSE), although they are now known as prion diseases. The occurrence of scrapie preceded recognition of other prion diseases of mammals and, in retrospect, it is the archetype of prion disorders (20). Prion diseases are defined by the accumulation of an abnormal isoform (PrPSc) of the host-encoded protein (PrPC) in the central nervous system (CNS) in all cases. The abnormal protein may also be detected in other tissues such as in the lymphoreticular system (LRS) and variably in other tissues/body fluids.

An atypical form of scrapie was first described in Norway in 1998 (5). Subsequently, active surveillance using rapid immunochemical methods has provided evidence for the widespread occurrence of this atypical form of scrapie (also known as Nor98), throughout Europe, with reports of cases also in the Falkland Islands (10) and North America (24). Although the epidemiology is not suggestive of transmission in the field (5) this type of scrapie can be transmitted experimentally (31) Retrospective studies have now identified British cases from the late 1980s, predating active surveillance (13). Atypical scrapie has been identified in sheep of genotypes considered to be resistant to classical scrapie, and in goats (2, 29).

The interaction of agent variables (particularly strain) and host variables determines the disease phenotype. In sheep, different PrP genotypes are associated with relative susceptibility to TSEs (17). Polymorphisms at codon 171 are of particular significance in determining overall susceptibility of sheep to classical scrapie, while variations at 141 and 154 affect susceptibility of sheep to the more recently identified atypical form of disease (5, 26, 28).

Characterisation of different strains of scrapie isolates has, historically, relied upon transmission to rodents (biological strain typing) (7), principally using inbred (wild-type) mice, but increasingly also a number of different transgenic mouse constructs (18). Molecular TSE-typing uses differential epitope binding of PrPSc in immunohistochemistry (IHC) or Western immunoblotting (21). The ability to distinguish scrapie from bovine spongiform encephalopathy (BSE) is of particular importance in small ruminants because of the zoonotic nature of the latter and the potential for past exposure of small ruminants to the feed-borne BSE agent. However, the mechanisms by which strain and host parameters influence disease phenotype are still incompletely understood.

Because of the known inadequacies of baseline (passive) surveillance and the absence of active surveillance components, the true scrapie status of many countries is unknown, and it is probably impossible to establish freedom from infection in a national flock without recourse to disproportionate levels of active surveillance. Some countries have never recorded the disease against a background of general and/or targeted surveillance, while others have maintained freedom for various periods through rigorous preventative policies and monitoring. The disease usually occurs in sheep 2–5 years of age. Rarely are cases present in sheep less than 1 year of age. In atypical scrapie, significant numbers of cases have been reported in sheep over 5 years of age. In some instances, the commercial lifespan of the sheep may be too short or exposure has occurred too late in life to allow the clinical disease to develop. Classical scrapie has also been described in goats, and captive moufflon (Ovis musimon), a primitive type of sheep. Most breeds of sheep are affected. The infection in sheep may be passed from ewe to lamb in the period from parturition to weaning. Infection can also pass horizontally to unrelated sheep or goats, especially when parturition occurs in confined areas. Fetal membranes are known to be a source of infection, and milk has recently been shown to be infectious to lambs (23). Pasture previously grazed by, or buildings previously inhabited by, infected sheep may also represent a risk. Animals incubating the disease, and even animals that never develop clinical signs, may still be a source of infection to others.

The biohazard for humans from scrapie diagnostic testing appears to be limited, but appropriate precautions should be taken. Creutzfeldt-jakob disease (CJD) has been found to occur at no greater frequency in those with occupations providing closest contact with the agent, but the extreme chemical and physical resistance of the scrapie agent and the fact that it is experimentally transmissible by injection to a wide spectrum of mammalian species suggest the prudence of preventing human exposure.

B. DIAGNOSTIC TECHNIQUES

1. Identification of the agent

The specific causative agent has never been isolated or described for any prion disease. Characterisation is therefore based on the identification of phenotypic parameters, such as clinical signs, through histopathological profiles, to increasingly complex immunochemical and biological parameters.
The clinical signs of scrapie (35) usually start insidiously, often with behavioural changes that are evident only from repeated inspections. These subtle presenting features, which may include apparent confusion, separation from the flock and a staring gaze, progress to a more definite neurological illness, frequently characterised by signs of pruritus and ataxia or incoordination of gait. Either the pruritus or the ataxia usually emerges to dominate the clinical course. Death may occur after a protracted period of only vague neurological signs or may even occur without premonitory signs. These clinical signs, individually, are not disease-specific and clinical suspicion of disease should be confirmed by further testing.

Pruritus is recognised principally by compulsive rubbing or scraping against fixed objects, nibbling at the skin and scratching with a hind foot or horns. This results in extensive loss of wool, particularly over the lateral thorax, flanks, hindquarters and tail head. The persistence of pruritus often results in localised self-inflicted skin lesions. These may occur in areas of wool loss and on the poll, face, ears and limbs. A characteristic ‘nibble reflex’ can often be elicited by scratching the back, and may also be evoked by the sheep’s own scraping movements. Some sheep or goats with scrapie, however, may not present with evident signs of pruritus.

Ataxia or incoordination of gait may first become apparent as difficulty in positioning the hind limbs on turning, swaying of the hindquarters and a high stepping or trotting gait of the forelimbs. Stumbling and falling occur, but the sheep is generally able to quickly regain a standing posture. These signs progress to weakness and recumbency. Information on the phenotypic variants of scrapie, termed atypical scrapie, or Nor98 suggests clinical features being dominated by pruritus in the absence of pruritus; circling has also been observed (6, 22, 26, 30, 35). Other signs of scrapie may include teeth grinding (bruxism), low head carriage, a fine head or body tremor and, rarely, seizures or visual impairment. In most cases, there is also a loss of bodily condition or weight.

Progress of the clinical disease is very variable, lasting for a week or up to several months, with an inevitably fatal outcome. There is also variation in the clinical signs among individual animals and in different breeds of sheep. These variations may be due to the influence of host genotype and strain of agent. Environmental factors may also have an influence on the disease course. The clinical diagnosis of individual cases of scrapie can therefore be difficult. The clinical signs may, especially in the early phase of the disease, resemble those of some other conditions of adult sheep, including ectoparasitism, pseudorabies (Aujeszky’s disease), rabies, cerebral listeriosis, ovine progressive pneumonia (maedi-visna), pregnancy toxaemia (ketosis), hypomagnesaemia and chemical and plant intoxications.

Video-clips illustrating the clinical signs of scrapie can be viewed on the website of the OIE Reference Laboratory at Veterinary Laboratories Agency (VLA) (http://www.vla.gov.uk/science/sci_tse_rl_video.htm), which is also the European Commission TSE Community Reference Laboratory (11). Other historical videotape footage of classical scrapie signs can also be sourced (36). Video-clips of atypical scrapie signs are also available (22). Full narratives describing the clinical signs of scrapie are available in the literature (5, 22, 35).

According to the prion hypothesis, demonstration of PrPSc would constitute identification of the agent, but, by definition, transmission from infected tissues, usually to laboratory rodents by injection, is the only available means of detection of infectivity. Long incubation periods and the failure of some natural scrapie sources to transmit to specific mice strains mean that it is impractical to use the criterion of transmissibility for diagnosis. However, biological characterisation on transmission is an important experimental component of the definition of any emerging new phenotypic variants of scrapie and for discriminatory approaches to distinguish cases of scrapie from BSE in sheep or goats (11). Given the absence of any specific gross pathological changes, the laboratory diagnosis of classical scrapie (15) has, in the past, been reliant on the demonstration of histopathological changes, notably vacuolation, in the CNS. Prior to the routine use of immunochemical detection of PrPSc, morphological demonstration of PrPSc in the form of scrapie-associated fibrils (SAF) was employed as an adjunct to histopathological diagnosis. SAF are visualised in unfixed brain extracts using negative-stain electron microscopy and have also been recovered from formaldehyde-fixed and autolysed brain tissue (8, 32).

The histopathological diagnosis was historically based on examination of a single section of medulla oblongata taken at the level of the obex, considered the predilection site for morphological vacuolar changes (38). This approach is still valid for the confirmation of classical scrapie, but it will not detect atypical scrapie. However, PrPSc detection using IHC examination and/or Western immunoblotting techniques, performed on the medulla oblongata, have increased the efficiency of the detection methods, and this has now extended to the active surveillance of large populations using rapid PrP detection tests (see below). Detectable PrPSc precedes vacuolation and clinical signs, making the immuno-based tests a more sensitive option (19). While clinically suspect cases of scrapie should, where suitable samples are available, continue to be investigated initially by histopathological examination for morphological changes, diagnostic criteria must now include the demonstration of PrPSc in the CNS. The medulla remains the most consistent and appropriate diagnostic level of the CNS for classical scrapie, however the accumulation pattern of PrPSc in atypical scrapie is different (5, 25). In atypical scrapie, the medulla shows only minimal change, while much more consistent and overt lesions can usually be identified in the cerebellum, thalamus and basal ganglia. Therefore, taking practical and logistical sampling considerations into account the medulla and cerebellum should both be examined as a minimum for robust diagnosis. This approach assists in the characterisation of the prion protein that is present, and contributes to
discrimination of disease phenotypes, particularly between classical and atypical scrapie, and indeed is also sufficient for the discrimination of scrapie and BSE (3, 39).

Some commercially available rapid methods for the detection of PrPSc, introduced originally for the diagnosis of BSE, are also approved for scrapie diagnosis (12). These rapid tests take the form of Western immunoblot, luminescence (LIA)– or enzyme-linked immunosorbent assay (ELISA)-based methods, and provide preliminary screening from which positive or inconclusive results are subject to examination by confirmatory IHC or Western blot methods. All these methods have been shown to be able to detect classical scrapie in the appropriate samples (12), but some have subsequently been shown to detect atypical scrapie to a varying degree or not at all. The analytical sensitivity of these kits is kept under review by the European Commission, and links to information on the performance of currently approved tests can be found on the Reference Laboratory webpages (11).

Failure to observe characteristic histological changes or to detect disease-specific PrP/SAF does not confirm the absence of the disease; agreement between the results of multiple diagnostic approaches provides the best assurance of accuracy. Clearly, in surveillance situations where monitoring is for the purpose of obtaining evidence of freedom from scrapie in small ruminant species, it may be necessary to apply multiple diagnostic criteria and to use at least two laboratory methods (histopathological and IHC, or immunoblotting) on accurately sampled CNS tissue (minimum medulla and cerebellum) to maintain a high degree of confidence in negative results.

Passive surveillance of scrapie, comprising the examination of CNS material from clinically suspect cases, has, in recent years, been complemented in many countries by active surveillance, targeting healthy adult culls and fallen stock (diseased or dead animals, also called risk animals) screened at post-mortem using the rapid test methods. In scrapie, the opportunity also exists for screening approaches that do not rely solely on examination of the CNS tissue from dead animals to detect exposed animals, but uses the widespread presence of PrPSc in lymphoreticular tissue in many animals to enable demonstration of infected animals by biopsy of palatine tonsil, nictitating membrane, superficial lymph nodes or, most recently, rectal mucosa lymphoreticular tissue (16). Any of these tissues can usefully be included in the screening of culled populations too, to maximise disease detection. It must be noted that not all animals with classical scrapie have detectable lymphoreticular involvement (1, 16), and no PrPSc has yet been detected in the lymphoreticular tissues of cases of sheep or goats with atypical scrapie (6). However, the testing of lymphoreticular tissue offers the opportunity to detect some animals infected with classical scrapie at relatively early stages of incubation, before the CNS is positive.

Whereas large-scale testing, to determine freedom from scrapie, should include targeted examination of peripheral tissues as well as the CNS of younger animals, surveillance for prevalence of disease could potentially limit tissue examination to the CNS of adult sheep and goats for the reasons given above. However, testing to estimate disease prevalence needs to take into account a number of factors, including the stratification of the sheep-farming industry, dose or level of infection within particular flocks, frequency of disease and relative involvement of the LRS in different genotypes, and the effect of genotype/agent strain combination on incubation period.

The need to distinguish between cases of scrapie and possible BSE in sheep and goats has required the development of diagnostic methods with the potential to discriminate between the agents causing these infections. Studies suggest that the conformation of disease-specific PrP produced in BSE-infected sheep is different from that of disease-specific PrP found in natural sheep scrapie (13, 21, 34). These conformational differences may be detected by immunoblotting or IHC techniques using epitope-specific antibodies. Within the European Union, the strategy for this distinction comprises examination of source CNS material after initial detection through active or passive surveillance (initial screening), in a primary, secondary and tertiary phase procedure (11), involving a Western immunoblot method capable of such discrimination, followed by peer review and further investigation by biochemical and IHC methods, and finally, if necessary, mouse transmission to a standard panel of wild-type mice (see Chapter 2.4.6 BSE). Interpretation of the in-vitro methods (Western immunoblot or ELISA) is reliant on differences between BSE and scrapie in the N-terminal cleavage site for Proteinase-K digestion of PrPSc. The in-situ IHC approach relies on distribution and epitope-specific labelling patterns of PrPSc in brain and lymphoreticular tissues. Increasingly, for biological characterisation of agent strain, appropriate transgenic mouse constructs are being used, although comprehensive data comparing models are still sparse.

Quality control (QC), quality assurance (QA) and appropriate positive and negative control samples are essential parts of testing procedures and advice and control materials can be requested from the OIE Reference Laboratories (39).

a) Sample preparation

Concerns regarding BSE in small ruminant populations and, the recognition of atypical scrapie, have influenced the strategies for sampling and diagnosis. Although comprehensive sampling and multiple testing methods would provide the most robust contingencies for these and possible future uncertainties in the diagnosis of prion diseases of small ruminants, operational factors also determine what is practically and
economically possible. The relative implementation of passive and active surveillance programmes, and the
diagnostic methods applied, further influence sampling strategy. Selection and recommendation of methods
is therefore under constant review.

For routine diagnosis, the sampled CNS material is either stored fresh or frozen for subsequent biochemical
tests or is fixed for histological preparations. Where programmes are in place to identify possible infections
with BSE in small ruminant populations, all sampling should be conducted aseptically, using new sterile
disposable instruments, or instruments sterilised under conditions specified for the decontamination of
prions, (see Chapter 2.4.6 BSE). Cross contamination at necropsy/sampling should be avoided. Thus, in the
following procedures where fresh tissue is sampled for biochemical methods, an aliquot should, if required,
be reserved for transmission studies. Although in many instances disease can be confirmed on autolysed or
suboptimally preserved material, such samples can only provide limited evidence of the absence of scrapie.

Sheep in which clinical disease is suspected (passive surveillance) should be killed by intravenous
injection of barbiturate and the whole brain removed by standard necropsy procedures as soon after death
as possible. Whole brain removal is advisable to allow pathological examinations for differentiation between
possible different TSE phenotypes and differential diagnosis of non-TSE brain disorders. Methods of
subdividing the brain tissue, for application of PrP-detection techniques requiring fresh tissue and for
histological techniques, are dependent on the optimum sensitivities of each of the tests for different brain
areas and the compromise that precisely the same area cannot be used for both fresh/frozen and fixed
tissue approaches. The following protocol is suggested but may be subject to modification to satisfy the
particular portfolio of tests. Further information can be obtained from OIE Reference Laboratories (11, 39).

Initially, a coronal block of medulla oblongata inclusive of the obex (see Chapter 2.4.6 BSE, Figure 1) is
taken for fixation into at least 10 times its volume of 10% formol saline and held for 3–5 days prior to
trimming and histological processing to paraffin wax for histopathological and IHC examinations. Care should
be taken to ensure that this sample is not frozen. For the detection of PrP (or SAF), fresh tissue samples are
taken and stored frozen (–20°C or below) prior to extraction of protein. Samples should, if possible, provide 5
g of tissue. This should be taken initially from the caudal medulla and, if necessary supplemented with
brainstem immediately rostral to the medulla – obex sample. Subdivision of this tissue to accommodate
multiple biochemical methods can be achieved by hemisecting in the median plane or by transverse
sectioning. Possible variation in sampling for rapid test requirements at the level of the obex is dealt with
below in the discussion on active surveillance approaches. Where the whole brain is available additional
fresh samples are advocated to minimise false-negative diagnoses, taking into account the possibility that
there may be strain-specific targeting of other parts of the brain. For example, in atypical scrapie, cerebellum, thalamus and basal ganglia regions provide the optimum sites for testing (5, 25).

The remaining brain tissue is fixed in approximately 10 times its volume of 10% formal saline for at least
1 week and then cut transversely as required to obtain blocks for histological processing to paraffin wax. The
initial sampling of the single block of the medulla may well be sufficient for IHC and the morphological
diagnosis (see Chapter 2.4.6 BSE, Fig. 1). Requirements for pathological characterisation or differential
diagnosis can be fulfilled by taking additional areas of the brain stem and, as necessary, representative
blocks of all major brain regions. Sections 5 µm in thickness, are stained with haematoxylin and eosin and
examined initially for the morphological changes and, as required, for IHC detection of PrPSc, as outlined
below.

For active surveillance programmes, methods for removal of the brainstem via the foramen magnum
using proprietary spoon-shaped instruments, similar to those employed in cattle for sampling for BSE
diagnosis (see Chapter 2.4.6 BSE, Figure 2) have been devised for sheep. Although not advisable, the
approach can also be used for clinical suspect cases. The minimum sampling is the brainstem at the level of
the obex and, if it is required to also diagnose atypical scrapie, the cerebellum. The brainstem portion is
either hemisectioned in the median plane to provide half (fresh/frozen) for a rapid test and half (fixed) for
histopathology. Alternatively a complete coronal slice inclusive of the obex is fixed and a similar adjacent
caudal medulla slice taken for the rapid test. The complete coronal slice has been recommended in the past,
to establish the symmetry of morphological changes, but with the use of rapid molecular techniques there is
competition between tests for the optimal diagnostic sites at the obex. Some rapid test kits use a core
sampling approach to obtain an appropriate mass of material from the obex region. While hemisectioning of
the obex region of the brainstem will result in loss of the ability to assess vacuolar lesion symmetry, the
greater specificity provided by IHC to detect PrPSc largely offsets this disadvantage. However if this, or a
core sampling approach, is adopted, it becomes critical to ensure that the contralateral target site is not
compromised. The dorsal nucleus of the vagus nerve (the optimal target area for cases of classical scrapie)
is a narrow column and lies close to the midline (see Chapter 2.4.6 BSE, Figure 3). The options are also
dependent on the specific sampling instruments provided by the test kit manufacturer. For all sampling
methods it is vital that operators are trained and that the training includes instruction in the gross and cross-
sectional neuroanatomy of the brainstem and the precise location of the target areas for disease-specific
PrPSc accumulation.
For differentiation of classical and atypical cases, portions of cerebellum are required fixed and fresh/frozen.

b) Histological examination

Morphological changes in the CNS are those of a spongiform encephalopathy comprising principally vacuolation of neuronal cell bodies and the surrounding neuropil, accompanied by a variable and usually less conspicuous gliosis (particularly an astrocytic reaction). Typically, the lesions have a bilaterally symmetrical distribution. There is considerable variation in the distribution pattern of vacuolation and other changes, but lesions, at least in classical scrapie, are usually most apparent in the brain stem and frequently affect the dorsal nucleus of the vagus nerve. Care must be taken if interpreting histopathology alone, as some incidental vacuolation of neurons may also be present in the brains of apparently healthy sheep, albeit at a low frequency (37). There is no direct correlation between the severity of clinical signs and pathological changes (4, 14). A clinical diagnosis of suspected scrapie cannot be refuted by a failure to find significant vacuolar changes in the brain. The absence of lesions is therefore not evidence of the absence of scrapie infection, as this can exist without either clinical signs or detectable morphological changes. This is particularly relevant for atypical scrapie, in which there is no vacuolation in the brainstem. In these cases, vacuolation, if it occurs at all, is generally restricted to the molecular layer of the cerebellar cortex, the cerebral cortex and the basal ganglion.

Despite such reservations, the histological examination of sections of medulla oblongata at the obex may be sufficient to confirm a diagnosis, in most cases, of clinically suspect classical scrapie (15, 38). Clearly, the absence of lesions can be established with greatest confidence by examining a number of areas representative of the whole brain.

c) Detection of disease-specific forms of PrP

Methods for the demonstration of accumulation of disease-specific forms of PrP in specified target areas now provide the principal approach to the diagnosis of natural scrapie (15). In suspect clinical cases the combined use of IHC and Western immunoblotting is advocated to confirm the diagnosis. IHC on tissue sections to demonstrate accumulation of PrP$^{\text{Sc}}$ should be carried out in parallel with routine histology in suspected cases. Combined use of IHC and Western immunoblotting is also recommended where histological lesions are mild in severity and considered equivocal. In active surveillance programmes, the primary diagnosis will usually be accomplished using rapid test methods and, in the case of positive or inconclusive results, confirmatory methods should also be applied. Test methods are detailed on the web sites of OIE Reference Laboratories (see Chapter 2.4.6 BSE). A wide range of antisera and monoclonal antibodies for PrP detection by immunochemical methods are now in use and some are commercially available.

- Immunohistochemical methods

Disease-specific PrP$^{\text{Sc}}$ in scrapie-affected brain is demonstrated by IHC on routinely formalin-fixed material by the application of a variety of epitope demasking techniques and the use of appropriate antibodies to PrP (11, 39). Recognition of morphological disease-specific immunolabelling configurations, their cellular associations and neuroanatomical distribution patterns provide the basis for a confirmatory diagnosis in classical (27) and in atypical (5) scrapie. The method used at the European TSE Community Reference Laboratory, VLA, is provided in the following link (http://www.defra.gov.uk/vla/science/docs/sci_tse_rl_prp_ihc.pdf). In recognition of the distribution of generic skills in national reference laboratories, and the power of the IHC approach, variation in methodology is possible from laboratory to laboratory, subject to appropriate proficiency testing and quality assurance.

If histopathological examination and IHC results cannot be achieved, e.g. because of autolysis (owing to the poor state of the sample, i.e. severely autolysed cases), then immunoblotting, the ELISA methods and SAF detection are the remaining test options available. Similarly, these methods can also be applied in circumstances when, sometimes in error at necropsy, CNS material intended for fixation and histological examination has been frozen. IHC methods can still be applied to such samples if they are subsequently fixed, but the ability to identify anatomical sites may be compromised, meaning that any ‘negative’ result must be qualified. With modification, the method for SAF detection may also be applied successfully to formalin-fixed tissue (8). Because of its lower diagnostic sensitivity, SAF detection should not be used as a sole test if an immunochemical method is possible.

- Western immunoblot methods

All Western immunoblotting techniques rely on detergent extraction followed by treatment with proteinase K enzyme to digest any normal host protein (PrP$^{\text{c}}$). This leaves only PrP$^{\text{Pres}}$ (the truncated, partially protease-resistant form of the abnormal prion protein [PrP$^{\text{Sc}}$]) to be bound by a specific antibody, which provides a detection system in positive brain samples (see also Chapter 2.4.6 BSE). A diagnosis based on the detection of PrP$^{\text{Pres}}$ by Western immunoblotting for classical scrapie cases requires that a wide region of three immunostained bands corresponding to proteins of molecular mass 19 kDa (unglycosylated PrP$^{\text{res}}$) to 32 kD
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(diglycosylated PrPres) be present in the proteinase-K-treated scrapie sample lanes only, and that control sample lanes provide appropriate comparisons. Several sensitive Western immunoblotting methods for the detection of ovine classical scrapie PrPres have been published (2, 3, 33).

For atypical scrapie cases four protein bands are visualised by Western immunoblotting, ranging from 19 kDa (unglycosylated PrPres) to 27 kDa (diglycosylated PrPres) with a fourth band below 15 kDa. The techniques used to detect atypical scrapie employ a reduced concentration of proteinase K enzyme in the procedure (2, 3, 5).

The original technique used for diagnosis of BSE, which has been referred to as ‘the OIE Western immunoblotting technique’ (11, 33, 34) relies on detergent extraction of large amounts of fresh brain material (nominally 4 g) followed by ultracentrifugation to concentrate the PrP and finally the proteinase K treatment is applied. This technique will also detect classical and atypical scrapie samples.

- Rapid test methods

Rapid immunodiagnostic test formats for the detection of PrPSc in small ruminant brain tissue have been developed and have been evaluated for diagnostic use (12), and these are all commercially available. Reference should be made to instructions provided by commercial manufacturers, which will have been subject to approval before use, and subsequent quality assurance. Deviation from test methods provided by commercial manufacturers is not normally permitted, and not recommended.

The rapid tests rely on the optimisation of the reagents used for extraction and digestion and specific antibodies for detection, which negates the need for lengthy ultracentrifugation steps. The tests require fresh medulla taken at the obex or just caudal to the obex. Most rapid tests use less that 0.5 g of material and many sampling tools are designed to sub-sample precise amounts. However, to allow for possible additional testing at least 1 g of initial sample is advised. Some laboratories use the OIE Western immunoblotting technique (if enough tissue is available) to confirm any weak-positive samples that are initially detected using a rapid test (11). The increased amount or concentration of PrPres extracted by ultracentrifugation from the larger aliquot of brain tissue can give improved sensitivity. Rapid test methods have been used to identify atypical scrapie cases (13) and detection is optimised if the appropriate brain sample (such as cerebellum) is tested.

Prospects for more sensitive diagnostic tests for scrapie and other TSEs are mainly directed at present on the refinement of existing methods and the development of new approaches to the detection of disease-specific forms of PrP. Achievement of the consistent performance of rapid test methods for the primary diagnostic approach is paramount, particularly with regard to the capacity of tests to recognise both classical and atypical scrapie phenotypes. Overall diagnostic sensitivity is strongly influenced by the accuracy of sampling.

d) Other diagnostic tests

As for BSE of cattle (see Chapter 2.4.6 BSE) tests that can be applied effectively to the live animal to detect scrapie cases in the early stage of incubation remain elusive, despite several avenues of research. The pursuit of non-prion protein biomarkers, including possibly through metabolomic or proteomic approaches, may offer prospects but there are constraints, including that the key tissue is accessible and that specificity is demonstrable.

2. Serological tests

A serological immune response to the scrapie agent has not been detected.

3. Genetic screening

Scrapie control and elimination strategies based on genetic selection for resistance to classical scrapie have been deployed successfully in some countries. Selection is made on determination of the common polymorphisms of the sheep PrP gene. As an aid to the control of classical scrapie: breeding stock, particularly rams, of appropriate PrP genotype can be selected to produce progeny with reduced risk of developing disease (9). Such genotyping services are available on a commercial basis in North America and in several countries in Europe. The test is performed using DNA extracted from white blood cells obtained from ethylene diamine tetra-acetic acid (EDTA)-treated blood samples. (Other tissue such as skin [e.g. ear punches] can also be used, as can other tissues such as brain, for screening cull population samples.) Selection of breeding stock can be based on the most scrapie-resistant animals, homozygous for arginine at codon 171, thereby reducing the incidence of classical scrapie in individual flocks. However, these animals are not always common in flocks, and in some breeds the genotype is actually absent.

A strategic approach to eliminating scrapie infection from national sheep flocks or geographical regions by adopting a national genetic breeding programme is possibly premature. The shortage of sheep that are
homzygous for arginine at codon 171 is one factor. The lack of data on the effects of a high prevalence of such a genotype on productivity, resistance to diseases other than scrapie and viability in general is another factor. Furthermore, there is, as yet, insufficient knowledge concerning the prevalence and epidemiology of atypical scrapie, although early indications are that an alternative breeding strategy would be required for this form of the disease. Decisions on the appropriateness of such programmes must take account of a thorough evaluation of the current national/regional/local scrapie situation, the availability of replacement resistant sheep, the sheep importation policy, availability of testing facilities and the desirability and support of the sheep industry, especially the willingness of sheep breeders to commit themselves to the programme for a long period of time.

**C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS**

There are no biological products available.

**REFERENCES**


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NB: There are OIE Reference Laboratories for Scrapie (see Table in Part 3 of this Terrestrial Manual or consult the OIE Web site for the most up-to-date list: www.oie.int).