CHAPTER 3.7.11.

SCRAPIE

SUMMARY

Description of the disease: *Scrapie is a neurodegenerative disease of sheep and goats. So-called 'atypical' scrapie is clinically, pathologically, biochemically and epidemiologically unrelated to 'classical' scrapie, is likely to be non-contagious and a spontaneous degenerative condition of older sheep and rarely of goats. This chapter describes tests for both conditions so that they may be differentiated.*

Classical scrapie is characterised by vacuolar changes in the central nervous system (*CNS*)*. It has been recognised as a clinical disorder for more than 250 years, and is classified as a transmissible spongiform encephalopathy* (*TSE*), *or prion disease, as defined by the accumulation of an abnormal form of a host membrane glycoprotein* (*prion protein or PrP*) *referred to as* PrP^{Sc}, *in the CNS. In animals of some genotypes,* PrP^{Sc} *accumulation is also detectable in lymphoreticular tissues. Polymorphisms of the PrP gene are associated with susceptibility to scrapie. Breeding for resistance has been used as a tool in the control of ovine classical scrapie, but no genotype appears to be completely resistant to infection.*

The more recently identified condition known as atypical scrapie has some clinical and pathological features similar to classical scrapie but is not considered to be transmitted in field situations. The epidemiology is consistent with a condition that occurs sporadically. Consequently surveillance for classical scrapie will detect occasional cases of atypical scrapie. It has been reported in sheep with PrP genotypes that are relatively resistant to classical scrapie.

Classical scrapie is endemic in many countries, where it has often been introduced by importation. Australia and New Zealand have maintained freedom from classical scrapie through strict restrictions on imports and other measures. Classical scrapie may be transmitted from dam to offspring in the period from parturition to weaning, and potentially in utero. *It can also be transmitted horizontally to unrelated sheep or goats. The infectious material can persist on pastures and in buildings. Fetal membranes are a source of infection, and milk from clinically affected animals can transmit disease. The incubation time between primary infection and clinical disease is usually longer than 1 year and may sometimes exceed the commercial lifespan of the animal. The majority of cases occur between 2 and 5 years of age. Clinical disease develops only if the agent enters the CNS. Atypical scrapie, where it presents clinically, is reported mostly in older animals, and occurs with a geographical distribution suggestive of a spontaneous disease, although it has been transmitted experimentally.*

Identification of the agent: *The classical disease may be recognised by the clinical signs, which are variable but usually start insidiously with behavioural abnormalities that progress to more obvious neurological signs including pruritus and incoordination. Affected animals have poor body condition. Atypical scrapie cases may present with ataxia. Diagnosis is confirmed by demonstration of vacuolation or the immuno-detection of* PrP^{Sc} *in target areas within the brain. Immuno-detection of* PrP^{Sc} *in brain samples forms the basis of the rapid tests, which are most often used in active surveillance programmes. In experimental studies in sheep and goats,* PrP^{Sc} *accumulation in the brain is not detectable until several months after challenge, so a negative test result does not necessarily equate to an uninfected animal.*

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

Currently recognised forms of scrapie can be transmitted with variable efficiency to a range of wild type and PrP transgenic laboratory rodents by inoculating them with infected brain tissue, but long incubation times preclude this as a practical diagnostic procedure.

***Serological tests:** Scrapie infection is not known to elicit any specific immune response and so there are no diagnostic tests to detect specific antibodies.*

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

A. INTRODUCTION

Classical scrapie (also known as la tremblante; Traberkrankheit; Gnubberkrankheit; Prúrigo lumbar) 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. There is no evidence of a causal link between classical or atypical scrapie and human TSEs. It is the archetype of the transmissible spongiform encephalopathies (TSEs). Prion disorders with similar pathology have been found to occur naturally in several species including man (Hörnlimann, 2006). They are defined by the consistent accumulation of an abnormal isoform (PrP^{Sc}) of the host-encoded protein (PrP^{C}) in the central nervous system (CNS), and variable PrP^{Sc} detection in other tissues such as in the lymphoreticular system (LRS) and other tissues/body fluids.

Atypical scrapie (also known as Nor98), is also a neurodegenerative disease of sheep and goats first described in Norway in 1998 (Benestad *et al*., 2008). Like the classical disease it is associated with abnormal prion protein accumulations, however unlike classical scrapie it has not been shown to be naturally transmitted to in contact animals under field conditions. Active surveillance using rapid immunochemical methods has provided evidence for its widespread occurrence throughout Europe, with reports of cases also in the Falkland Islands (Epstein *et al*., 2005), North America (Loiacono *et al*., 2009), Australia and New Zealand (Kittelberger *et al.,* 2010). Although the epidemiology is not suggestive of transmission in the field (Benestad *et al*., 2008) and it is not considered transmissible from an animal health perspective it can be transmitted experimentally (Simmons *et al.,* 2011). Retrospective studies have identified cases from the 1980s, predating active surveillance. Atypical scrapie has been identified in sheep of genotypes considered to be relatively resistant to classical scrapie, and in goats.

Various disease-specific isoforms of the abnormal prion protein (PrP^{Sc}) are now widely regarded to be the causal agents in prion diseases. Disease characterisation is based on a range of host phenotypic parameters, such as clinical signs, histopathological profile and immunopathology, PrP^{Sc} biochemical characteristics such as protease sensitivity and cleavage site and, if necessary, biological parameters in rodent models.

1. Clinical signs

The clinical signs of classical scrapie (Konold & Phelan, 2014) 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 animal is generally able to quickly regain a standing posture. These signs progress to weakness and recumbency. 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.

In atypical scrapie the clinical features are dominated by ataxia in the absence of pruritus; circling has also been observed.

Video-clips illustrating the clinical signs of scrapie can be viewed on the webpages of TSE European Union Reference Laboratory, TSE-LAB-NET (http://www.tse-lab-net.eu/).

Progression 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 small ruminants, including ectoparasitism, pseudorabies (Aujeszky's disease), rabies, cerebral listeriosis, ovine progressive pneumonia (maedi-visna), pregnancy toxaemia (ketosis), hypomagnesaemia and chemical and plant intoxications.

2. Host genetic factors

Agent strain and host variables determine the expression of disease. In sheep, different PrP genotypes are associated with relative susceptibility to TSEs. Polymorphisms at codons 136 and 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 atypical scrapie. In goats, PrP genotype also influences susceptibility to disease. The mechanisms by which strain and host parameters influence disease phenotype are still incompletely understood. (See EFSA [2014] for recent overview.)

3. Strain characterisation

Strain characterisation historically relied upon rodent bioassay. The ability to distinguish scrapie from bovine spongiform encephalopathy (BSE) is of particular importance in small ruminants because of BSE's zoonotic nature and the potential for past exposure of small ruminants through contaminated feed. For this purpose, molecular typing uses differential epitope binding of PrP^{Sc} in immunohistochemistry (IHC) or Western immunoblotting.

4. Surveillance

Because of the known inadequacies of baseline (passive) surveillance and the absence of active surveillance components, the classical scrapie status of many countries is unknown. Objectively to establish freedom from infection in a national flock requires sustained and substantial levels of active surveillance. Some countries have never recorded classical scrapie against a background of general and/or targeted surveillance, while others have maintained freedom for various periods through rigorous preventative policies and monitoring. Classical scrapie usually occurs in sheep 2–5 years of age. Rarely are cases present in sheep less than 1 year old. In atypical scrapie a significant proportion of cases have been reported in sheep over 5 years of age. Rarely, sheep have been identified with mixed infections of classical and atypical scrapie. In some instances with classical scrapie, the commercial lifespan of the sheep may be too short or exposure has occurred too late in life for the clinical disease to develop. Classical and atypical scrapie have also been described in goats, and classical scrapie in captive moufflon (*Ovis musimon*). Most breeds of sheep are affected. Classical scrapie may be transmitted from dam to offspring in the period from parturition to weaning, and potentially *in utero* (Spiropoulos *et al.,* 2014) Infection can also pass horizontally to unrelated animals, even without direct contact (Dexter *et al.,* 2009). Fetal membranes are known to be a source of infection, and milk can also transmit disease (Konold *et al*., 2013). Pasture previously grazed by, or buildings previously inhabited by, infected animals also represent a risk (Gough *et al.,* 2015; Hawkins *et al.,* 2015). Animals incubating the disease, and even animals that never develop clinical signs, may still be a source of infection to others.

5. Biosafety

The biohazard for humans from scrapie diagnostic testing appears to be limited, but laboratory manipulations with potentially contaminated material must be performed at an appropriate biosafety and containment level determined by biorisk analysis (see Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*). Although Creutzfeldt-Jakob disease (CJD) of humans has been found to occur at no greater frequency in those with occupations providing closest contact with the scrapie agent, 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 including humanised transgenic mice (Cassard *et al.,* 2014) and non-human primates (Comoy *et al.,* 2015) suggests the prudence of preventing human exposure.

B. DIAGNOSTIC TECHNIQUES

Table 1. *Test methods available for diagnosis of scrapie and their purpose*

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification						
Histopathology	n/a	n/a	–	+	–	n/a
IHC	n/a	n/a	++	+++	++	n/a
Western immunoblot	n/a	n/a	++	+++	++	n/a
Rapid tests	n/a	n/a	+++	+	+++	n/a

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limit its application; – = not appropriate for this purpose; n/a = not applicable.
Although not all of the tests listed as category +++ or ++ have undergone formal validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.
IHC = immunohistochemistry.

A disease-specific partially protease-resistant, misfolded isoform (PrP^{Sc}) of a membrane protein PrP^{C}, has a critical importance in the pathogenesis of TSE. According to the prion hypothesis, PrP^{Sc} is the principal or sole component of the infectious agent, and confirmation of the diagnosis is reached by the application of immunohistochemical (IHC) or immunochemical detection of PrP^{Sc} in brain tissue. By definition, specific demonstration of infectivity would rely on experimental transmission, but ethical considerations and the long incubation periods associated with TSEs mean that the criterion of transmissibility is not used for routine 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.

The laboratory diagnosis of classical scrapie relies primarily on the immunodetection of PrP^{Sc}, although more traditional histopathology methods can still be used to detect vacuolar lesions in the CNS (Gavier-Widen *et al*., 2005) Histopathological examination, historically based on examination of a single section of medulla oblongata taken at the level of the obex, (the earliest consistent neuroanatomical site for morphological vacuolar changes [Wood *et al*., 1997]) is still valid for the confirmation of classical scrapie, but it will not detect atypical scrapie (Moore *et al.,* 2008). However, PrP^{Sc} detection using IHC examination or immunodetection techniques, performed on the medulla oblongata, have increased diagnostic sensitivity, and the active surveillance of large populations is now undertaken using rapid PrP^{Sc} detection tests (see below). Detectable PrP^{Sc} precedes vacuolation and clinical signs, making the immuno-based tests a more sensitive option. 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 PrP^{Sc} in the CNS. The medulla remains the most consistent and appropriate diagnostic level of the CNS for classical scrapie, however the accumulation pattern of PrP^{Sc} in atypical scrapie is different (Benestad *et al*., 2008; Moore *et al*., 2008).

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 and classification.

Some commercially available rapid methods for the detection of PrP^{Sc}, introduced originally for the diagnosis of BSE, are also approved for scrapie diagnosis and others have been specifically developed and approved for use on small ruminant samples. These rapid tests take the form of Western immunoblot or enzyme-linked immunosorbent assay (ELISA)-based methods, and provide preliminary screening from which samples giving positive or inconclusive results are subject to examination by confirmatory IHC or Western immunoblot methods. All these methods have been shown to be able to detect scrapie in the appropriate samples (EFSA, 2005a; EFSA, 2012). 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 TSE-LAB-NET. A list of those tests that are currently approved for use by the European Commission can be found in Annex X of Regulation (EC) No. 999/2001 as last amended.

Failure to observe either characteristic histological changes or to detect PrP^{Sc} does not confirm the absence of the disease; agreement between the results of multiple diagnostic approaches provides the best assurance of accuracy. In surveillance for the purpose of obtaining evidence of freedom from scrapie in small ruminant populations, 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 classical 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 classical 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 PrP^{Sc} 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 (Gonzalez *et al*., 2006). However it must be noted that not all animals with classical scrapie have detectable lymphoreticular involvement and PrP^{Sc} has not yet been detected in the lymphoreticular tissues of cases of sheep or goats with atypical scrapie (Benestad *et al*., 2008). 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.

Due to the complex epidemiology of scrapie, the part of the population that should be targeted for sampling, as well as the tissues to be analysed, differs with the different purposes of the testing. Surveillance for prevalence of the disease could 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. The conformation of disease-specific PrP produced in BSE-infected sheep is different from that of disease-specific PrP found in natural sheep scrapie. These conformational differences may be detected by immunoblotting or IHC techniques using epitope-specific antibodies (summarised in EFSA [2005b]). 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 involving a Western immunoblot method capable of such discrimination followed by peer review and further investigation by biochemical and IHC methods of any cases in which primary discrimination was equivocal and, finally, if classification still cannot exclude BSE, mouse transmission to a standardised panel of transgenic mice as described in the EURL Discriminatory Testing Handbook, available on TSE-LAB-NET. 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 PrP^{Sc}. The *in-situ* IHC approach relies on distribution and epitope-specific labelling patterns of PrP^{Sc} in brain and lymphoreticular tissues. Newer *in-vitro* methods, such as quaking-induced conversion (QuIC) (Orrù *et al.,* 2012), and protein misfolding cyclic amplification (PMCA) (Castilla *et al.,* 2006) are increasingly being used to increase diagnostic sensitivity, although none have been formally approved for statutory purposes at present. These methods use normal PrP as a substrate, and multiple rounds of protein aggregation to achieve amplification of even very small amounts of PrP^{Sc}, PMCA in particular also demonstrates some potential for agent strain discrimination (Gough *et al.,* 2014).

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.

1. Identification of the agent

1.1. Specimen selection and 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 practical and economical. 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 3.4.5 *Bovine spongiform encephalopathy*). Cross contamination at necropsy/sampling should be avoided. Thus, in the following procedures where fresh tissue is sampled for biochemical methods, an aliquot should be reserved for transmission studies. Although in many instances disease can be confirmed on autolysed or suboptimally preserved material, such samples can provide only limited evidence of the absence of scrapie.

1.1.1. Suspect clinical cases

Sheep in which clinical classical or atypical scrapie is suspected (detected by 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 examination for differentiation among possible different manifestations of prion disease and the differential diagnosis of non-prion associated brain disorders. Methods of sampling the brain tissue for application of PrP-detection techniques requiring fresh tissue and for histological techniques are based on knowledge of the diagnostic 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 recommended but may be subject to modification to satisfy any particular portfolio of tests. Further information can be obtained from OIE Reference Laboratories (consult the OIE Web site for the most up-to-date list).

Initially, a coronal block of medulla oblongata inclusive of the obex (see chapter 3.4.5, 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 processing for histopathological and IHC examinations. Care should be taken to ensure that this sample is not frozen. For the detection of PrP^{Sc}, fresh tissue samples are taken for immediate testing or stored frozen (–20°C or below) prior to extraction of protein. Specimens 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. Subsampling 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 section on sampling for rapid tests. Where the whole brain is available additional fresh specimens 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 optimal sites for testing, not the medulla (Benestad *et al*., 2008; Moore *et al*., 2008).

The remaining brain tissue is fixed in approximately 10 times its volume of 10% formol saline for at least 1 week and then cut transversely 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 3.4.5, 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 PrP^{Sc}, as outlined below.

1.1.2. Sampling for active surveillance using rapid tests

For the application of rapid tests, 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 3.4.5, Figure 2) have been devised for sheep. Although not ideal, the approach can also be used for clinical suspect cases. The minimum specimen tissue required is the brainstem at the level of the obex. To detect atypical scrapie the cerebellum should also be sampled with a spoon via the foramen magnum following removal of the brainstem. 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 PrP^{Sc} offsets this

disadvantage. However if this, or a core sampling approach, is adopted, it is 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 that lies close to the midline (see chapter 3.4.5, 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 PrP^{Sc} accumulation.

For differentiation of classical and atypical cases, portions of cerebellum are required fixed and fresh/frozen.

1.2. 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. In classical scrapie lesions are usually most apparent in the brainstem 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 (Hörnlimann, 2006). There is no direct correlation between the severity of clinical signs and pathological changes. A clinical diagnosis of suspected scrapie cannot be refuted by a failure to find significant vacuolar changes in the brain, and the examination should be supported by tests to detect the accumulation of disease-specific forms of PrP. 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 this variability the histological examination of sections of medulla oblongata at the obex may be sufficient, in most cases, to confirm a diagnosis of clinically suspect classical scrapie (Gavier-Widen *et al*., 2005; Wood *et al*., 1997). The absence of lesions can be established with greatest confidence by examining a number of areas representative of the whole brain.

1.3. 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 both classical and atypical scrapie (Gavier-Widen *et al*., 2005). 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^{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. A wide range of antisera and monoclonal antibodies for PrP detection by immunochemical methods are now in use and some are commercially available. Advice on test methods and reagents is available from the OIE Reference Laboratories for scrapie, and further information is available on their web sites (see chapter 3.4.5).

1.3.1. Immunohistochemical methods

Disease-specific accumulation of PrP^{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 against PrP. Recognition of morphological disease-specific immunolabelling configurations, their cellular associations and neuroanatomical distribution patterns provide the basis for a confirmatory diagnosis in classical (Ryder *et al*., 2001) and in atypical (Benestad *et al*., 2008) scrapie. As an example, the method used at the European Union Reference Laboratory for TSE, and a list of antibodies proven to be of use for IHC, is provided in the following link http://www.tse-lab-net.eu/documents/tse-oie-rl-prp.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. owing to the poor state of the sample, (i.e. severely autolysed cases), then immunoblotting and the rapid test methods are the remaining test options available. Similarly, these methods can also be applied in circumstances where, 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 Western immunoblot detection may also be applied successfully to formalin-fixed tissue (Kunkle *et al*., 2008)

1.3.2. 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^{c}). This leaves only PrP^{res} (the truncated, partially protease-resistant form of the abnormal prion protein [PrP^{Sc}]) to be bound by a specific antibody, which provides a detection signal in positive brain samples. A diagnosis based on the detection of PrP^{res} by Western immunoblotting for classical scrapie cases requires that immunolabelled bands corresponding to proteins within a range of molecular mass from 17 kDa (unglycosylated PrP^{res}) to 27 kD (diglycosylated PrP^{res}) 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 PrP^{res} have been published (Arsac *et al*., 2007; Stack, 2004; Stack *et al.,* 2006).

For atypical or Nor98 scrapie cases, multiple bands are visualised by Western immunoblotting ranging from approximately 11 to 31 kD. As atypical scrapie PrP^{Sc} is less resistant to proteinase K digestion than classical scrapie PrP^{Sc}, the techniques optimised to detect atypical scrapie employ a reduced concentration of this enzyme in the procedure (Arsac *et al.,* 2007; Benestad *et al*., 2008).

The original technique used for diagnosis of BSE, which has been referred to as 'the OIE Western immunoblotting technique' (Stack *et al.,* 2006) relies on detergent extraction of large amounts of fresh brain material (nominally 2–4 g) followed by ultracentrifugation to concentrate the PrP and finally the proteinase K treatment is applied. This technique can also detect classical and atypical scrapie samples.

Detailed protocols for Western immunoblotting can be found on TSE-LAB-NET.

1.3.3. Rapid test methods

Rapid immunodiagnostic tests for the detection of PrP^{Sc} in small ruminant brain tissue have been developed and have been evaluated for diagnostic use (EFSA, 2005a; 2012), 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 without assessment and documentation (see Validation Recommendation Chapter 2.5.8 *Comparability of assays after changes in a validated test method*).

The rapid tests rely on the optimisation of the reagents used for extraction and digestion and specific antibodies for detection. The tests require fresh brain tissue, which to maximise diagnostic sensitivity for classical scrapie, should be brainstem taken at the obex or just caudal to the obex. To ensure maximum diagnostic sensitivity for atypical scrapie, cerebellum should also be tested. Most rapid tests use less than 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. If enough tissue is available, some laboratories use the Western immunoblotting technique (see Section B.1.3.2 above) to confirm any weak-positive samples that are initially detected using a rapid test. The increased amount or concentration of PrP^{res} extracted by ultracentrifugation from the larger aliquot of brain tissue can give improved sensitivity.

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, as well as BSE in small ruminants. Overall diagnostic sensitivity is strongly influenced by the accuracy of sampling.

1.4. Other diagnostic tests

As for BSE of cattle (see chapter 3.4.5) 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 accessibility of tissue to be tested and specificity of the test method. Methods using *in-vitro* protein amplification are proving very sensitive for the detection of some prion diseases (Castilla *et al.,* 2006; Orru *et al.,* 2012), but have not yet been formally evaluated for application within statutory surveillance systems, although some have been successfully piloted for surveillance applications in humans (Lacroux *et al.,* 2014; Orru *et al.*, 2014).

2. Serological tests

An antibody response to the scrapie agent has not been detected and so serological tests are not feasible.

3. Genetic screening for resistance

Scrapie control and elimination strategies based on genetic selection for resistance to classical scrapie in sheep 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 (recently reviewed in EFSA 2014). 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, i.e. animals of genotypes which encode alanine on both alleles at codon 136, arginine on both alleles at codon 154 and arginine on both alleles at codon 171 (so called ARR/ARR animals), 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 breeding programme selecting ARR/ARR sheep will not however ensure resistance to atypical scrapie. Decisions on the appropriateness of such programmes must take into account 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

ARSAC J.N., ANDREOLETTI O., BILHEUDE J.M., LACROUX C. BENESTAD S.L. & BARON T. (2007). Similar biochemical signatures and prion protein genotypes in atypical scrapie and Nor98 cases, France and Norway. *Emerg Infect Dis*., **13**, 58–65.

BENESTAD S.L., ARSAC J-N., GOLDMANN W. & NÖREMARK M. (2008). Atypical/Nor98 scrapie: properties of the agent, genetics and epidemiology. *Vet. Res.,* **39**, 19.

CASSARD H., TORRES J.M., LACROUX C., DOUET J.Y., BENESTAD S.L., LANTIER F., LUGAN S., LANTIER I., COSTES P., ARON N., REINE F., HERZOG L., ESPINOSA J.C., BERINGUE V. & ANDRÉOLETTI O. (2014). Evidence for zoonotic potential of ovine scrapie prions. *Nat. Commun*., **5**, 5821.

CASTILLA J., SAA P., MORALES R., ABID K., MAUNDRELL K. & SOTO C. (2006). Protein misfolding cyclic amplification for diagnosis and prion propagation studies. *Methods Enzymol*, **412**, 3–21.

COMOY E.E., MIKOL J., LUCCANTONI-FREIRE S., CORREIA E., LESCOUTRA-ETCHEGARAY N., DURAND V., DEHEN C., ANDREOLETTI O., CASALONE C., RICHT J.A., GREENLEE J.J., BARON T., BENESTAD S.L., BROWN P. & DESLYS J.P. (2015). Transmission of scrapie prions to primate after an extended silent incubation period. *Sci. Rep.*, **5**, 11573. doi: 10.1038/srep11573.

DEXTER G., TONGUE S., HEASMAN L., BELLWORTHY S., DAVIS A., MOORE S.J., SIMMONS M.M., SAYERS R., SIMMONS H.A. & MATTHEWS D. (2009). The evaluation of exposure risks for natural transmission of scrapie within an infected flock. *BMC Vet. Res*., **5**, 38

EPSTEIN V., POINTING S. & HALFACRE S. (2005). Atypical scrapie in the Falkland Islands. *Vet. Rec.,* **157**, 667–668.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA) (2005a). Evaluation of rapid post-mortem TSE tests intended for small ruminants. *EFSA Scientific Report*, **49**, 1–16.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA) (2005b). Opinion on classification of atypical transmissible spongiform encephalopathy (TSE) cases in small ruminants. *EFSA J.,* **276**, 1–30.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA) (2012). Scientific Opinion on the evaluation of new TSE rapid tests submitted in the framework of the Commission Call for expression of interest 2007/S204-247339. *EFSA J.,* **10**, 2660

EUROPEAN FOOD SAFETY AUTHORITY (EFSA) (2014). Scientific Opinion on the scrapie situation in the EU after 10 years of monitoring and control in sheep and goats. *EFSA J.,* **12**, 3781

GAVIER-WIDEN D., STACK M.J., BARON T., BALACHANDRAN A. & SIMMONS M. (2005). Diagnosis of transmissible spongiform encephalopathies in animals: a review. *J. Vet. Diagn. Invest.,* **17**, 509–527.

GONZALEZ L., DAGLEISH M.P., BELLWORTHY S., SISÓ S., STACK M.J., CHAPLIN M.J., DAVIS L.A., HAWKINS S.A.C., HUGHES J. & JEFFREY M. (2006). Postmortem diagnosis of preclinical and clinical scrapie in sheep by the detection of disease-associated PrP in their rectal mucosa. *Vet. Rec.*, **158**, 325–331.

GOUGH K.C., BAKER C.A., SIMMONS H.A., HAWKINS S.A. & MADDISON B.C. (2015). Circulation of prions within dust on a scrapie affected farm. *Vet. Res*., **46**, 40.

GOUGH K.C., BISHOP K. & MADDISON B.C. (2014). Highly sensitive detection of small ruminant bovine spongiform encephalopathy within transmissible spongiform encephalopathy mixes by serial protein misfolding cyclic amplification. *J. Clin. Microbiol*., **52**, 3863–3868.

HAWKINS S.A., SIMMONS H.A., GOUGH K.C. & MADDISON B.C. (2015). Persistence of ovine scrapie infectivity in a farm environment following cleaning and decontamination. *Vet. Rec.,* **176**, 99.

HÖRNLIMANN B. (2006). Prions in Humans and Animals, Hörnlimann B., Riesner, D. & Kretzschmar H., eds. de Gruyter, Berlin, Germany.

KITTELBERGER R., CHAPLIN M.J., SIMMONS M.M., RAMIREZ-VILLAESCUSA A., MCINTYRE L., MACDIARMID S.C., HANNAH M.J., JENNER J., BUENO R., BAYLISS D., BLACK H., PIGOTT C.J. & O'KEEFE J.S. (2010). Atypical scrapie/Nor98 in a sheep from New Zealand. *J. Vet. Diagn. Invest*., **22**, 863–875.

KONOLD T., MOORE S.J., BELLWORTHY S.J., TERRY L.A., THORNE L., RAMSAY A., SALGUERO F.J., SIMMONS M.M. & SIMMONS H.A. (2013). Evidence of effective scrapie transmission via colostrum and milk in sheep. *BMC Vet. Res.,* **9**, 99.

KONOLD T. & PHELAN L. (2014). Clinical examination protocol to detect atypical and classical scrapie in sheep. *J. Vis. Exp.*, **83**:e51101. doi: 10.3791/51101.

KUNKLE R.A., NICHOLSON E.M., LEBEPE-MAZUR S., ORCUTT D.L., SRINIVAS M.L., GREENLEE J.J., ALT D.P. & HAMIR A.N. (2008). Western blot detection of PrP Sc in archived paraffin-embedded brainstem from scrapie-affected sheep. *J. Vet. Diagn. Invest.*, **20**, 522–526.

LACROUX C., COMOY E., MOUDJOU M., PERRET-LIAUDET A., LUGAN S., LITAISE C., SIMMONS H., JAS-DUVAL C., LANTIER I., BÉRINGUE V., GROSCHUP M., FICHET G., COSTES P., STREICHENBERGER N., LANTIER F., DESLYS J.P., VILETTE D. & ANDRÉOLETTI O. (2014). Preclinical detection of variant CJD and BSE prions in blood. *PLoS Pathog.,* **10**:e1004202.

LOIACONO C.M., THOMSEN B.V., HALL S.M., KIUPEL M., SUTTON D., O'ROURKE K., BARR B., ANTHENILL L. & KEANE D. (2009). Nor98 scrapie identified in the United States. *J. Vet. Diag. Invest*., **21**, 454–463.

MOORE S.J., SIMMONS M.M., CHAPLIN M.J. & SPIROPOULOS J. (2008). Neuroanatomical distribution of abnormal prion protein in naturally occurring atypical scrapie cases in Great Britain. *Acta Neuropathologica,* **116**, 547–559.

ORRÚ C.D., BONGIANNI M., TONOLI G., FERRARI S., HUGHSON A.G., GROVEMAN B.R., FIORINI M., POCCHIARI M., MONACO S., CAUGHEY B. & ZANUSSO G. (2014). A test for Creutzfeldt-Jakob disease using nasal brushings. *N. Engl. J. Med*., **371**, 519–529.

ORRÙ C.D., WILHAM J.M., VASCELLARI S., HUGHSON A.G. & CAUGHEY B. (2012). New generation QuIC assays for prion seeding activity. *Prion*, **6**, 147–152. doi: 10.4161/pri.19430.

RYDER S.J., SPENCER Y.I., BELLERBY P.J. & MARCH S.A. (2001). Immunohistochemical detection of PrP in the medulla oblongata of sheep: the spectrum of staining in normal and scrapie-affected sheep. *Vet. Rec*., **148**, 7–13.

SIMMONS M.M., MOORE S.J., KONOLD T., THURSTON L., TERRY L.A., THORNE L., LOCKEY R., VICKERY C., HAWKINS S.A.C., CHAPLIN M.J. & SPIROPOULOS J. (2011). Experimental oral transmission of atypical scrapie to sheep. *Emerg. Infect. Dis*., **17**, 848–854.

SPIROPOULOS J., HAWKINS S.A., SIMMONS M.M. & BELLWORTHY S.J. (2014). Evidence of *in utero* transmission of classical scrapie in sheep. *J. Virol*., **88** (8), 4591–4594. doi: 10.1128/JVI.03264-13. Epub 2014 Jan 22.

STACK M.J. (2004). Western Immunoblotting Techniques for the Study of Transmissible Spongiform Encephalopathies. *In*: Techniques in Prion Research, Lehmann S. & Grassi J., eds. Birkhäuser Verlag, Switzerland, 97–116.

STACK M., JEFFREY M., GUBBINS S., GRIMMER S., GONZALEZ L., MARTIN S., CHAPLIN M., WEBB P., SIMMONS M., SPENCER Y., BELLERBY P., HOPE J., WILESMITH J. & MATTHEWS D. (2006). Monitoring for bovine spongiform encephalopathy in sheep in Great Britain, 1998–2004. *J. Gen. Virol*., **87**, 2099–2107.

WOOD J.L.N., MCGILL I.S., DONE S.H. & BRADLEY R. (1997). Neuropathology of scrapie: a study of the distribution patterns of brain lesions in 222 cases of natural scrapie in sheep, 1982–1991. *Vet. Rec.,* **140**, 167–174.

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NB: There are OIE Reference Laboratories for Scrapie
(see Table in Part 4 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list:
http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on
diagnostic tests and reagents for scrapie

NB: FIRST ADOPTED IN 1989; MOST RECENT UPDATES ADOPTED IN 2016.