# CLINICAL SIGNS OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES IN SHEEP

**Warning:** This document links directly to video clips ranging in size from 690 kb to 1.9 Mb. The latter could take over 15 minutes to download using a standard modem connection. For more information about the content and the size of the video clips please see the TSE video clips page.

### Introduction

Inoculation of brain tissue from animals affected by transmissible spongiform encephalopathies (TSE) into mice is the historical method to distinguish different strains of scrapie [1]. More recently, strains have been characterised by the regional distribution of disease-associated prion protein ($PrP^d$) using immunohistochemistry and by the characteristic protein pattern of tissue samples using Western immunoblotting methods [2]. This has led to a new classification system of scrapie, which distinguishes classical scrapie as it has been known for centuries from atypical scrapie, which appears to affect older animals with a genotype associated with increased resistance to classical scrapie [3]. In addition, sheep can be infected with the agent associated with bovine spongiform encephalopathy (BSE), which has its own characteristics that can be distinguished from scrapie by immunohistochemical [4] and Western immunoblot examination of the brain [5]. Although there may be overlap in the clinical picture of these TSE in sheep, each type is mentioned separately in the text below.

## CLASSICAL SCRAPIE

### Case Characteristics

Most classical scrapie cases have been diagnosed in 2-5 year old sheep but cases may occur in younger animals. Scrapie has been confirmed in a 6 months old lamb with clinical signs of scrapie in Iceland.

The susceptibility or resistance to scrapie is determined by the PrP gene and appears to be independent of sex and breed. Animals with the highest susceptibility to classical scrapie have a genotype that encodes QQ (glutamine) at codon 171 ($VV_{136}RR_{154}QQ_{171}$ or

$AA_{136}RR_{154}QQ_{171}$ in breeds that usually do not carry the VRQ allele), whilst animals with the PrP genotype $AA_{136}RR_{154}RR_{171}$ are genetically most resistant to classical scrapie [6][1]. Affected flocks usually experience only small numbers of clinical cases in any year, sometimes only single cases. More explosive outbreaks have been reported, but are relatively rare. Most cases have an insidious onset and progress slowly over several weeks. In some cases, clinical signs may be observed for up to 7 months. Early clinical signs may not be noticed, especially in flocks that are not closely monitored. It is unclear if acute or peracute scrapie cases exist that do not display premonitory signs of illness. In one survey, 16% of confirmed scrapie cases were found dead without exhibiting signs before death [7]. Scrapie cases without apparent signs of scrapie but widespread vacuolation in the brain have also been diagnosed in abattoir surveys.

**Clinical Signs**

Early clinical signs can be fairly unspecific and are thus most likely to be observed by someone familiar with the animal. Farmers may suspect early scrapie in sheep of a flock in which scrapie is endemic before neurological signs are evident due to their experience with the disease. These early clinical signs include weight loss and subtle behavioural changes.

**Changes in mental status, behaviour and activity**

These are often the earliest clinical signs to be noticed. Animals may separate themselves from the rest of the flock and may become more flighty when approached and thus more difficult to catch. Affected sheep may also appear hyper-alert, may become more excitable and may display exaggerated responses to external stimuli, such as sudden movements or noises, e.g. created by a passing tractor or a hand clap (see video). When left undisturbed, scrapie cases may appear somnolent or dull with a vacant gaze and lowered head. This may become more obvious and permanent in advanced scrapie cases but may only episodic in the early stage and thus only detectable through prolonged observations. Scrapie cases tend to spend more time lying down than healthy sheep and are usually more inactive during these periods. Social interaction seems to be reduced in scrapie-affected sheep [8].

[1] Three codons of the PrP gene are of main importance for scrapie susceptibility: 136 (A = alanine or V = valine), 154 (A = arginine or H = histidine) and 171 (Q = glutamine or R = arginine)

Teeth grinding is frequent in scrapie cases and occurs either spontaneous during periods of inactivity, whilst being handled or in combination with lip licking (see video).
Appetite is usually not affected until the very late stage of the disease although it has been reported that scrapie-affected sheep spent less time feeding on hay. In addition, mastication and bolus regurgitation may be reduced in sheep with scrapie [8,9]. Abnormal drinking patterns have also been reported in the early stages of scrapie. Sheep with scrapie may drink more frequently but only small amounts each time [10].
Frequent urination (pollakiuria) of small amounts of urine may be observed in some scrapie cases, which has been attributed to hyperactive detrusor function and variation in the urethral tone [11].

**Pruritic activities**

Grooming behaviour seems to be increased in most sheep with scrapie. Scrapie cases spent more time rubbing on objects (e.g. fences), scratching, licking and self-nibbling. In the later stages, rubbing may be accompanied by lip licking and smacking ('self-induced nibble reflex'), which gives the sheep an expression of satisfaction. These pruritic activities are however often only displayed when the animal is undisturbed or accustomed to an observing person (see video).
Most scrapie cases respond to scratching of the back with lip licking and nibbling (similar to the self-induced nibble reflex; frequently but not always also with rapid extrusions of the tongue), which has been called 'nibble reflex'. In advanced cases of scrapie, the 'nibble reflex' may be elicited by simply touching the animal's back. As some cases, however, respond to the scratch test with only rhythmical head or body movements (curling, flexion or extension of the head and neck, or body swings) without nibbling lip movements we prefer to use the term 'positive scratch response' for any response that is repeatable and stereotypical [2] (see videos 1, 2, 3, 4). Although pruritus is a classical feature of scrapie, some cases may not display this sign, which led to the classification of 'ataxia type of scrapie' [12] or 'paralytic form of scrapie' [13]. A positive scratch response was more frequent in Irish sheep of the genotypes ARQ/ARH or ARQ/ARQ than in sheep of the genotype ARQ/VRQ [14].

[2] Stereotypy is defined as the persistent repetition of senseless acts. It may be persistent maintenance of a bodily attitude or repetition of senseless movements.

**Scratch test**

The test sites are displayed in Fig. 1. Test sites should be scratched repeatedly (at least 3 times) and with a pause between each stimulus. A repeatable, stereotypical response is a positive scratch response. The test requires a fairly quiet and immobile animal since a response may not be detected in excited animals. Ideally, handlers should stand next to the sheep and use their legs to 'press' the animal against a wall (see Fig. 2).

Most pruritic cases will be detected if animals are observed for signs of excessive grooming in combination with the scratch test, because some animals may not respond to the scratch test.

The scratch test is not specific for scrapie since pruritic skin conditions, e.g. mange, can cause the same response.

**Fig. 1 Scratch test sites response**

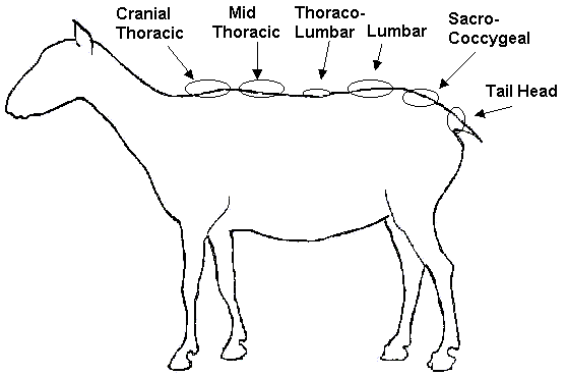
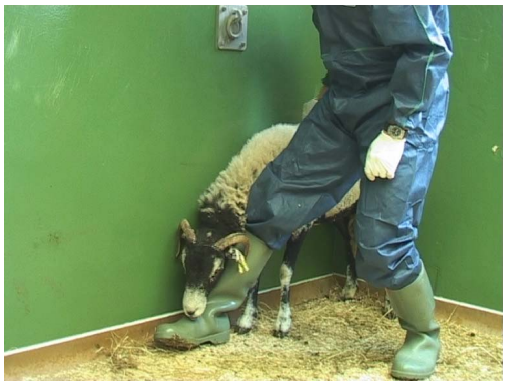



**Fig. 2. Testing the scratch**



Intense nibbling, rubbing and scratching will eventually lead to discolouration and loss of wool as well as hyperpigmentation and lesions of the skin. Skin lesions initially present as superficial abrasions, excoriation with crusts if allowed to heal and lichenification but may become secondary infected by skin pathogens (e.g. *Staphylococcus aureus, Dermatophilus congolensis*), which results in the formation of nodules and pustules. It is also possible to isolate parapox-virus (orf) from these skin lesions. Fig. 3-4 show skin alterations observed in scrapie cases.

**Fig. 3. Hyperpigmentation and lichenification**

**Fig. 4. Papules**

**Postural changes**

Scrapie cases may stand with their hind limbs placed in an abnormal position (e.g. wide-based stance); they may also stand with their hind quarters low and their hind limbs partially flexed in a ‘crouching’ posture [13,14].

**Gait changes**

Gait abnormalities are usually observed later in the course of the disease in cases that present with the ‘classical’ signs behavioural changes, pruritus and weight loss. Gait deficits are often enhanced upon exercise, on turns and when going over steps. The gait may initially have a stiff appearance. Ataxia, characterised by a wobbly, uncoordinated gait, is usually more frequent in the hind limbs (see video). Scrapie cases often bunny hop (hopping simultaneously with both hind limbs) when made to run. A high stepping, dressage-type gait (hypermetria) may be present, which led to its German name ‘Traberkrankheit’ (trotting disease). Severe gait deficits ultimately result in difficulty getting up and eventually recumbency.

**Involuntary movements**

Scrapie cases frequently exhibit a tremor of the head, in particular when stressed during handling (see video and also video ). This may later progress to generalised (whole body)

tremors. Some cases may display myoclonic muscular contractions [15,16]. Electromyographic studies have shown that tremors consist of two components that occur asynchronously: tetanic contractions of skeletal muscles and trembling of skin muscles [13].

Seizures may be seen in some cases of scrapie [17] and may be precipitated by handling and excitement [18].

Collapsing episodes, which may be a form of cataplexy-narcolepsy, have been occasionally observed (see video). Sheep may suddenly collapse for up to several minutes when stressed (e.g. during handling or when chased by a dog) and then regain their feet unaided [19,20].

**Weight loss and loss of condition**

Loss of weight and condition (see Fig. 5) despite an apparently maintained appetite is present in the majority of scrapie cases and can be a very early but unspecific clinical sign of scrapie [21]. It may progress slowly to emaciation in advanced scrapie cases [14].

**Fig. 5. Loss of condition**

# Other miscellaneous clinical signs

### Ptyalism and dribbling of ruminal fluid and salivation

Increased salivation may be present in some cases [17]. Dribbling of ruminal fluid and consequently staining of the wool around the nostrils, mouth and chin has been observed occasionally in scrapie cases ([14], see Fig. 6). This may be the result of swallowing deficits as reported in some scrapie cases [22].

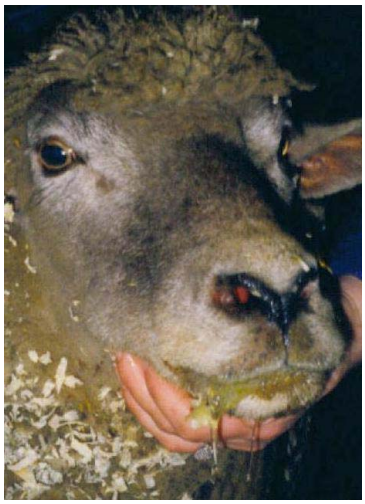


**Fig. 6. Dribbling of ruminal fluid**

### Ocular abnormalities

There are occasional reports of scrapie cases running into objects. Although this may be attributable to panic reactions in excitable animals, visual impairment has nevertheless been reported infrequently [23-25]. Other rare abnormalities include nystagmus [18,22], an absent menace reflex [14,21,26,27] (see video) or a reduced pupillary light reflex [13,21]. Hyper-reflective oval, blister-like areas throughout the tapetum lucidum were observed in two sheep with signs of visual impairment [26] but this has not been confirmed in more than 100 cases that were examined at VLA.

### Hypoaesthesia

Loss of sensation (hypoaesthesia or even anaesthesia) in the distal parts of the limbs was observed very early in cases of scrapie whilst loss of facial sensitivity may be observed in the late stages of the disease [21].

### Cardiac and respiratory abnormalities

Autonomic disturbances (cardiac arrhythmia, accompanied by tachycardia, bradycardia and intolerance to exercise) have been reported [21,28] although abnormalities in heart rate and rhythm were not observed in a separate study [14].

Scrapie cases may display an increased respiratory rate (see video) characterised by panting when stressed, e.g. after handling, or may show increased respiratory sounds. Scrapie has been diagnosed in a ewe that only displayed signs of respiratory disease [29]. It is important to note that sheep affected with scrapie may suffer from secondary infections, e.g. clostridial infections, pneumonia [30,31].

### Ruminal impaction

Gastrointestinal stasis has been reported in some scrapie cases [20,32].

### Milk Production

Hypogalactia and difficulty in milking is frequent in sheep that are used for milk production [33].

### Biochemical markers in body fluids

Some changes in blood neurotransmitters, hormones, metabolites, metals or immunoglobulins have been found in scrapie cases but it has so far not resulted in a test that would allow scrapie diagnosis in live animals. These changes included decreased blood serotonin [34], increased plasma 20beta-dihydrocortisol and urinary creatinine [35], increased concentrations of serum IgG [36], plasma growth hormone [37], blood copper and blood manganese [38]. Alterations in energy metabolism as determined by changes in the plasma concentrations of citrate, lactate and 3-hydroxybutyrate have also been reported [39].

Cerebrospinal fluid analysis was unable to distinguish scrapie cases from healthy sheep [20]. Other markers associated with neuronal damage in the brain, such as 14-3-3 protein detection in cerebrospinal fluid, have not been found useful for scrapie diagnosis [40].

## Electrodiagnostics

Abnormal electroencephalograms were obtained from a few sheep with scrapie [13,27]. Brainstem auditory evoked potentials and flash evoked visual potentials had reduced amplitudes in two confirmed scrapie cases [27]. Electrodiagnostic tests are expensive,

time-consuming and thus may not be of practical value but they may be useful to demonstrate lesions in certain neuroanatomical structures. However, they have been applied to only a very limited number of animals, and it is not known if these tests detect scrapie in sheep of various breeds and genotypes infected with different strains.

## Clinical diagnostic criteria

Based on the observation that scrapie cases display pruritus and ataxia, the absence of a nibble reflex alone or in combination with absent proprioceptive deficits was judged to be highly indicative of a scrapie-negative sheep on farms with reported cases of scrapie [41]. These criteria were proposed as an aid for scrapie surveillance in Italy.

A scoring system has been proposed to assess the clinical progression in classical scrapie based on the presence of eleven specific clinical signs: Hypoaesthesia in the limbs, body condition score of less than 3 (out of a maximum of 5 for an obese sheep) and alteration of mental status were given a value of 1, hyporeflexia in limbs, cardiac arrhythmia, pruritus/ wool loss and nibble reflex had a value of 2 and head tremor, hyperexcitability, ataxia/ gait abnormalities and teeth grinding had a value of 3 [21]. A different value was applied to different clinical signs to take into account the fact that certain clinical signs like teeth grinding, ataxia and tremor occur later in the disease. By adding the values of each sign if present the authors determined that a sum of 1-4 represented an early stage, a sum of 5-13 an intermediate stage and a sum of 14-23 a late stage of the disease. This scoring system has only been evaluated in Rasa aragonesa sheep with an ARQ/ARQ genotype.

Both clinical protocols require a close examination of sheep and familiarisation with neurological assessments of animals, which cannot always be expected from the keeper of sheep. It is important that farmers are able to recognise signs of disease, which can be variable as mentioned above, in order to report the disease to the local veterinary authority. The proposed clinical protocols may be useful to confirm the diagnosis of a suspect scrapie case reported by the farmer.

# ATYPICAL SCRAPIE

A new strain of scrapie that caused mainly ataxia in the absence of pruritus has first been described in Norway and was called Nor98. Disease-specific prion protein was found predominantly in cerebellar and cerebral cortices, and the molecular profile by Western immunoblot differed from that usually seen in scrapie (protein band of lower molecular mass than in previous scrapie cases) [42]. More of these cases have since been detected in other countries, and the disease was named “atypical scrapie” to distinguish it from the previously known scrapie disease, now called “classical scrapie” [3].

Most cases were detected in fallen stock or abattoir surveys; clinically affected animals have rarely been reported. Affected sheep are usually older, above 3.5 years of age. Major clinical signs are ataxia and behavioural changes, such as nervousness or anxiety, in addition to loss of weight or condition [42-44]. Affected sheep may also present with tremor and a deficient menace response (for video clips please refer to http://www.biomedcentral.com/1746-6148/3/2/additional/) [45]. Pruritus or physical changes suggestive of pruritus, such as wool loss, are usually absent (see Fig. 7) although a response (rhythmical head and body movements) to scratching of the back has been reported in one case [45].

Overall, there seem to be similarities to the ‘ataxic form’ of classical scrapie.

**Fig .7. Clinically affected Welsh Mountain sheep, with confirmed atypical scrapie and no evidence of wool loss.**

# BSE IN SHEEP

BSE has not yet been identified in naturally infected animals. Clinical signs of sheep with BSE have been described after experimental infection with the BSE agent using various routes.

An extreme short clinical course of less than a week was observed in intracerebrally and orally dosed Cheviots and the dominant signs were rapidly progressive ataxia and recumbency, whilst evidence of pruritus was observed in only one animal [46].

In a further experiment where Poll Dorset, Cheviot and Suffolk sheep were inoculated intracerebrally or intravenously, the clinical signs were comparable to ‘classical’ scrapie, although pruritus was less frequent and ataxia more frequent when compared to sheep experimentally infected with scrapie. Weight loss was present in only 11% of the animals [47].

The clinical signs after intra-peritoneal inoculation of one sheep were intense pruritus and ataxia that progressed over a period of 3 months [48].

Progressive clinical signs similar to scrapie, such as weight loss, pruritus with alopecia (see Fig. 8), tremors and weakness, have also been observed in orally infected ARQ homozygote Romney sheep [49].

**Fig 8 Orally infected Romney sheep with alopecia above the nostrils**

# DIFFERENTIAL DIAGNOSIS OF SCRAPIE

Scrapie may be confused with other neurological diseases or other diseases that cause a progressive loss of condition or pruritus with skin lesions. Scrapie usually causes neurological signs that are asymmetric or focal in distribution; diseases with asymmetrical neurological signs are less likely to be associated with scrapie. However, the presence of other diseases, e.g. pregnancy toxaemia or various skin diseases, does not rule out the absence of scrapie.

Tables 1-3 list the main differential diagnoses for scrapie. Diseases that generally occur in young animals (denoted as *) are listed because scrapie has been reported in a sheep of less than one year of age. Details on respective diseases can be found in veterinary textbooks [50,51].

**Table 1 Weight loss/ cachexia**

| Aetiology | Name of the Disease |
|---|---|
| Infectious | Leucosis, **paratuberculosis**, **pseudotuberculosis** (internal form), **endoparasites** |
| Nutritional | Protein/ energy malnutrition, cobalt deficiency |
| Miscellaneous | Dental disease, abomasal dilation and emptying defect |

**Table 2 Skin diseases**

| Aetiology | Name of the Disease |
|---|---|
| Infectious | **Mange**, parasitic dermatosis (e.g. lice infestation), cutaneous myiasis, dermatomycosis, **bacterial dermatitis** |
| Allergic | Allergic dermatitis |
| Miscellaneous | Photodermatitis |

**Table 3 Neurological diseases**

| Aetiology | Name of the Disease |
|---|---|
| Infectious | **Viral encephalomyelitides** (Aujeszki's disease, Borna disease, louping ill, maedi/visna, rabies), **bacterial meningoencephalo-myelitides and epidural abscesses** (listeriosis) and focal symmetrical encephalomalacia (*Clostridium perfringens*), **fungal meningoencephalo-myelitides** (aspergillosis, cryptococcosis – rare), **protozoan encephalomyelitis** (toxoplasmosis, sarcocystosis) and **cysts or parasitic migration** (*Coenurus cerebralis)* |
| Metabolic | **Pregnancy toxaemia, hypocalcaemia-hypomagnesaemia-complex, hepatic encephalopathy** |
| Congenital/ Anomalies | ovine congenital progressive muscular dystrophy*, ceroid lipofuscinosis*, cerebellar abiotrophy*, ovine GM1 gangliosidosis* |
| Neoplastic | Tumor (brain, spinal cord) |
| Nutritional | vitamin A deficiency, white muscle disease*, **cerebrocortical necrosis (CCN)***, copper deficiency (swayback, enzootic ataxia)*, ruminal acidosis |
| Toxic | Botulism, tetany, **various toxins** (mercury, lead, plant toxins, organophosphates) |
| Trauma | Spinal injury, verebral subluxation |
| Idiopathic | Humpy back, kangaroo gait, Swaledale encephalopathy, degenerative thoracic myelopathy |

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