## Review

# Current status of scrapie

**Claude Saegerman[1,*], Emmanuel Vanopdenbosch[2] and Dirk Berkvens[3]**

**Address:** [1] Department of Infectious and Parasitic Diseases, Epidemiology and Risk Analysis Applied to Veterinary Sciences, Faculty of Veterinary Medicine, University of Liege, Boulevard de Colonster, 20, B43b, B-4000 Liege, Belgium. [2] Department of Biocontrol, Veterinary and Agrochemical Research Centre, Groeselenberg 99, B-1180 Bruxelles, Belgium. [3] Department of Animal Health, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium.

***Correspondence:** Claude Saegerman. Email: Claude.Saegerman@ulg.ac.be

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## Abstract

Despite being controlled in many developed countries, scrapie remains endemic in many parts of the world. Economic losses result from mortality and culling of small ruminants and from market restrictions. Moreover, it is difficult to develop all-inclusive guidelines that could establish a scrapie-free status for a country. Unfortunately, the global picture remains incomplete because in many countries confusion still remains regarding the clinical picture of scrapie and information is not available owing to the absence of adequate epidemiosurveillance networks. Currently, the predominant theory is that $PrP^{Sc}$ is the infectious agent where host genetic factors play a central role. The precise transmission routes of scrapie and their relative contributions to the overall transmission intensity remain poorly documented and the physiopathology is not fully understood. However, it is evident that the purchase of female sheep from scrapie flocks, sharing pastures with scrapie flocks, sharing breeding rams and genetic host susceptibility are the main risk factors for the spread of the disease. A better understanding of the epidemiology of scrapie would greatly aid the development and evaluation of control and eradication strategies that were mainly based on selective depopulation of infected animals and genetically susceptible and/or related animals and also on the biosecurity and the use of selective genetic breeding programmes in healthy flocks. Some numbers of a new transmissible spongiform encephalopathy (TSE) form in small ruminants (atypical scrapie) have meanwhile been identified by TSE rapid testing using an assay, which also recognizes comparatively less proteinase K-resistant $PrP^{Sc}$. Uncertainties remain regarding the pathogenesis of this new TSE form, as well as regarding its potential transmissibility within the affected species and to other species. Thus far, no bovine spongiform encephalopathy (BSE) cases have been confirmed in sheep under natural conditions (a report of vertical transmission after experimental infection merits attention), but two historical cases of BSE in goats born in the 1990s have been identified. Currently BSE must also be considered in the differential diagnosis of scrapie. The development of prevention and control programmes should be assisted by new scientific findings.

**Keywords:** Scrapie, Spongiform encephalopathy, Prion disease, Sheep, Goats, Epidemiology, Disease control

## Background

Scrapie belongs to the transmissible spongiform encephalopathies (TSEs), which also include bovine spongiform encephalopathy (BSE) in cattle and human Creutzfeldt–Jakob disease (CJD) in humans. Scrapie is a fatal, chronic neurological disease that occurs mainly in domestic sheep (*Ovis aries*) and goats (*Capra hircus*) [1] and mouflons (*Ovis musimon*) [2]. Goats are rarely affected by scrapie [3]. Scrapie in sheep has been an endemic disease for over 250 years [1, 4]. Scrapie affects adult animals, with a peak age-at-onset of 2–5 years [3, 5]. Not a single clinical case of scrapie has been diagnosed in animals younger than six months [6]. The first description of the

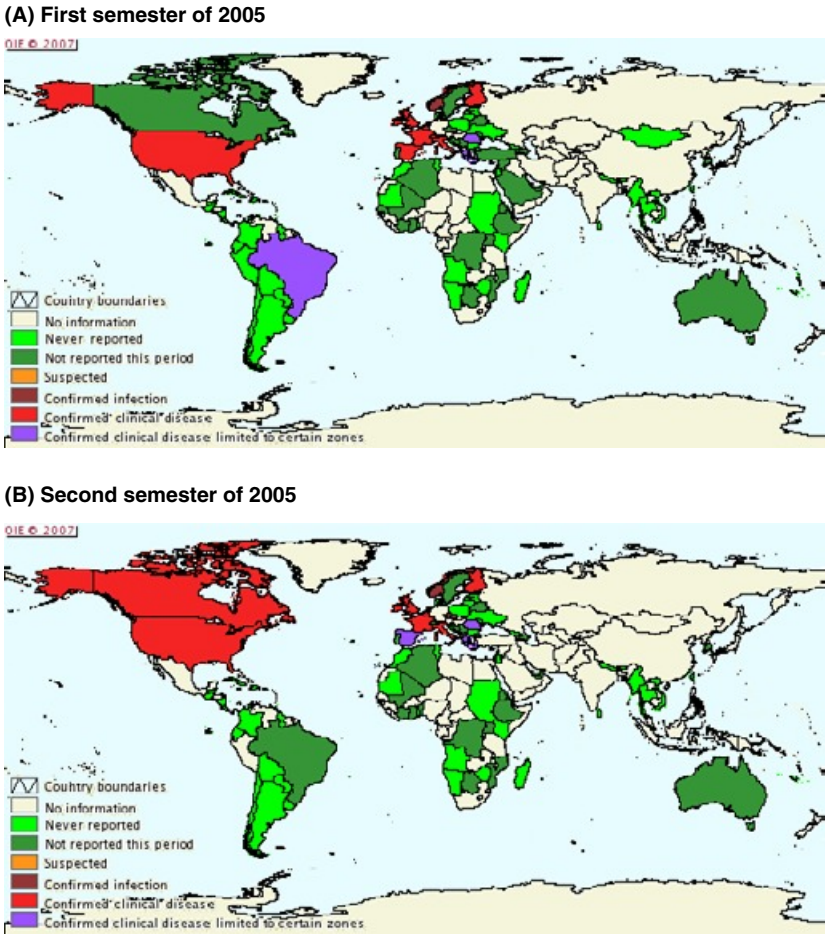


**Figure 1** Scrapie throughout the world in 2005 [16]

natural disease in goats dates back to 1942 [7]. Subsequently, only a few cases of scrapie have been reported in this species (e.g. [3, 8–11]). In France, a clinically suspected case of scrapie was described in a cow in 1883 but no brain-block was conserved [12]. This case is still an enigma. Currently, study of the aetiology of scrapie points to an infectious disease with a maternal and horizontal contagious transmission, where host genetic factors play a central role [9, 13, 14].

The emergence of BSE in cattle in the UK since 1986 sharply increased the interest in scrapie because of the theoretical proposal that BSE could have originated from sheep scrapie. However, a study investigating the pathogenicity for cattle, by intracerebral inoculation, of two pools a scrapie agents sourced in UK before and during the BSE epidemic has demonstrated that cattle inoculated with different pooled scrapie sources can develop different prion disease phenotypes, which were not consistent with the phenotype of BSE of cattle and whose isolates did not have the strain typing characteristics of the BSE agent on transmission to mice [15].

For this review we searched the term scrapie in the following databases: CAB Abstracts (http://www.cabi.org/) and PubMed (http://www.ncbi.nlm.nih.gov/). In addition we used the references from the articles obtained by this method to check for additional relevant material. We also checked the World Animal Health Information Database (WAHID) (http://www.oie.int/wahid-prod/public.php).

## Geographical and Temporal Distribution

### *Geographical Distribution*

The international zoosanitary situation for scrapie and its evolution in time can be found on the Web site of the International Organization for Animal Health (Office international de Epizooties, OIE) at the following address: http://www.oie.int/wahid-prod/public.php using the WAHID tool (Figure 1). The OIE member states update this database through the new World Animal Health Information System (WAHIS). More detailed data is also available from regional sources such as the report on the monitoring and testing of ruminants for the presence of TSEs in the European Union [17].

Despite being controlled in many developed countries, scrapie remains endemic in many parts of the world.

Unfortunately, the global picture remains incomplete because in many countries information is not available due to the absence of adequate surveillance networks. Nevertheless, the data that exist indicate that scrapie is more or less a worldwide problem.

It is difficult to develop all-inclusive guidelines which would establish scrapie-free status for a country. Currently, the criteria being used to establish a country's status are: quality of the national animal identification and movement tracing system for sheep and goats, mechanism and quality of reporting, diagnostic capability, past sourcing of sheep and goats, import restrictions and previous reports of scrapie in the country, the implementation of required control measures and thorough epidemiological investigations when detecting scrapie cases, and data on the distribution of scrapie resistance alleles within the major sheep breeds [18, 19]. Detailed criteria to establish a country as free of scrapie are available in the Terrestrial Animal Health Code at the following address: http://www.oie.int/eng/normes/mcode/en_chapitre_2.4.8.htm. Australian, New Zealand and Argentina are countries generally regarded as devoid of animal TSE [20].

### *Temporal Distribution*

The first reports of the existence of scrapie appear in eighteenth and nineteenth century literature from England and Germany [18], although there is evidence that it was present in northern Europe and Austro-Hungary before the beginning of the eighteenth century [21]. The earliest definite records of the occurrence of scrapie were in the UK in 1732 [22, 23] and in Germany in 1759 [24]. Throughout the 1700s and 1800s, scrapie was reported in many breeds of sheep in England as well as in continental Europe and later scrapie spread across the world, especially through the movement of sheep incubating disease (Table 1). An apparently important factor in the rapid spread of the disease during the eighteenth century was the practice of inbreeding with the aim of increasing wool production [21].

**Table 1** Occurrence of several scrapie outbreaks following the importation of infected animals (from [4, 18, 25])

| Year | Country | Reference |
|---|---|---|
| 1878 | Iceland | [26] |
| 1938 | Canada | [27] |
| 1947 | USA | [28] |
| 1952 | New Zealand | [29] |
| 1952 | Australia | [30] |
| 1958 | Norway | [1] |
| 1961 | India | [31] |
| 1963 | Belgium | [25] |
| 1964 | Hungary | [32] |
| 1966 | South Africa | [33] |
| 1970 | Kenya | [34] |
| 1973 | Germany | [35] |
| 1976 | Italy | [36] |
| 1977 | Brazil | [18] |
| 1979 | Yemen | [37] |
| 1988 | Sweden | [38] |
| 1988 | Cyprus | [39, 40] |
| 1990 | Japan | [41, 42] |

## Economic Losses

The economic impact of scrapie is mainly the result of individual producer losses within a marketing area and the loss of export markets for the entire country concerning live sheep, embryos, semen and other ovine products [4]. There is considerable variation in the type and severity of restrictions placed on imports because of scrapie (from a total import ban to simple certification with regards to the absence of clinical signs). Another economic factor is the cost of eradication or control programme (indemnity, compensation, human resources, diagnostic tests and record-keeping). Traditionally, producer losses were quantified by deaths from scrapie and a decrease in the value of breeding stock. Because scrapie is always fatal, mortality is closely linked with the flock incidence and this parameter shows considerable variation: from around 0.5–2% in some flocks (e.g. [43]) to around 20% in another (e.g. [44]). The use of rapid tests and the use of Bayesian approach offer a new opportunity to estimate the true prevalence at national level, but these results are still not available.

## Aetiology

Scrapie infectivity is associated with an abnormal form of a misfolded cell-surface sialoglycoprotein called $PrP^{C}$ coded by the host PrP gene [45]. The transition to the abnormal isoform ($PrP^{Sc}$ with 'sc' for scrapie) is a post-translational event and results from infection [4]. $PrP^{Sc}$ can be discriminated from $PrP^{C}$ by an increased β-sheet content, higher hydrophobicity and a partial resistance towards degradation by proteolytic enzymes ($PrP^{Sc}$). Cellular prion protein has a molecular mass of 27 kDa and is post-translationally glycosylated once (31 kDa) or twice (35 kDa) [46]. The molecular mechanism for the conformational change from $PrP^{c}$ to $PrP^{Sc}$ is unknown. Nevertheless, by using *in vitro* trials, metal ions such as copper and manganese have been shown to influence this conformational change [47, 48].

The physical and chemical properties of the scrapie agent are unusual: a considerable heat resistance, given the fact that residual infection was observed after an incineration at 600 °C using dry heat [49]; a high resistance to ionizing [50], ultraviolet and microwave irradiation [51, 52], all with the capacity to destroy nucleic acids necessary for virus replication. Traditional detergents and

**Table 2** The three major hypotheses about the nature of the scrapie agent (adapted from [179])

| Hypothesis | Nature of agent | Mode of replication | Main arguments for | Main arguments against | First reference |
|---|---|---|---|---|---|
| Prion | Protein only | Reverse translation, protein-directed protein synthesis or induction of host transcription | Recombinant prion protein is infectious [62] | Difficulty to take into account several strains and their variability [63–65] | [50] |
| Virino | Protein and nucleic acid; host-encoded protein with regulatory nucleic acid | Nucleic acid replicated by host enzymes with virino nucleic acid as the template | | Absence of specific nucleic acid [66] | [67] |
| Virus | Protein and nucleic acid; protein encoded by virus-specific nucleic acid | As per standard animal virus | $PrP^{Sc}$ and infectiosity are not associated [68] | Absence of immune response<br>Extreme physical and chemical resistances of agent<br>Absence of candidate virus | [69] |

disinfectants have only a partial neutralizing effect, formol even having the ability to fixate infectiousness [53]. On the other hand, the infective agent is sensitive to protein-denaturing procedures [50]. A combination of treatments leads to a better efficacy. More in particular, a one-hour immersion in 1 M soda or sodium hypochlorite (2% active chlorine), followed by porous-load autoclaving at 136 °C for 18 min, constitutes the standard inactivation and sterilization procedure for prions [54].

The exact origin of scrapie is still debated and the discussion will probably continue for many years to come. Initially, the debate focused on the genetic (e.g. [55]) or the infectious origin [9, 56–60]. Later on, three hypotheses for the nature of the scrapie agent were put forward: the proteinaceous infectious particle (prion) hypothesis ($PrP^{Sc}$ is the agent); the virino hypothesis ($PrP^{Sc}$ is part of the agent coupled to the agent genome) and the virus hypothesis (the agent is an unconventional virus with the protein coded by the viral genome) (Table 2). Currently, the predominant theory is that $PrP^{Sc}$ is the infectious agent [70] where host genetic factors play a central role [9, 13, 14, 44]. Recent results reinforce the role of scrapie agent as a full infectious agent [62].

## Physiopathology

The infection propagation pathway in the host requires an understanding of the host–pathogen interactions, which are determined by the diversity of scrapie strains and the host genetic susceptibility.

### *Strain of Scrapie*

Some strains of natural scrapie appear to attack genotypes differently. It has been observed that sheep of the same genotype, and breed, and from the same flock (environment) are susceptible to some strains of scrapie but resistant to others [18].

Scrapie strains can be differentiated based on several criteria, namely: clinical signs, incubation period, transmissibility, histopathology lesion profiles, inactivation behaviour, proteinase K (PK) resistance and cleavage site of $PrP^{Sc}$ and glycoprofile of $PrP^{Sc}$. Stable scrapie strains are able to change their characteristics after transmission to other species (e.g. mice), while instable strains can change their characteristics also in their original host. The mechanisms involved remain unknown and only hypotheses have been proposed, e.g. mechanism is an adaptive process (scrapie agent is determined at the molecular level) or a selection process (host is infected with a mixture of strains from which one strain was eventually selected) [63].

### *Genetic Host Susceptibility*

It has been known for about ten years that the susceptibility of sheep to natural (e.g. [13, 71–73]) and experimental (e.g. [74]) scrapie is influenced by amino acid polymorphisms at the positions 136, 154 and 171 of the prion protein (e.g. [75, 76]). In order to describe the sheep *PrP* genotypes, the protein allotype is given using the single letter code for each amino acid in numerical order: A – alanine, V – valine (codon 136); R – arginine, H – histidine (codon 154); R – arginine, Q – glutamine, H – histidine (codon 171) [73, 76, 77].

There are at least 20 different PrP alleles [78], including seven alleles at codons 136, 154 and 171, defining the haplotypes (in the following referred to as 'alleles') VRQ, ARQ, ARH, AHQ and ARR, that have been shown to be important for susceptibility to classical scrapie [72] (Table 3). The differentiation between classical and

**Table 3** The genotypes found in general population (random sampling) and in positive TSE cases in EU-25 during 2005 according to the NSP classification[1] [17]

| Classification | Resistance | Genotype | EU-25 sheep population (random sampling; *n*=191 699 animals) | TSE cases (*n*=897 animals) | |
|---|---|---|---|---|---|
| | | | | Atypical | Classical |
| NSP1 | Genetically most resistant | ARR/ARR | 15.7% | 8.9% | 0.3% |
| NSP2 | Genetically resistant | ARR/ARQ, ARR/ARH, ARR/AHQ | 43.5% | 43% | 1.5% |
| NSP3 | Genetically little resistance[2] | ARQ/ARQ | 33.1% | 22.8% | 44% |
| | | AHQ/AHQ, ARH/ARH, ARH/ARQ, AHQ/ARH, AHQ/ARQ | 7.4% | 24% | 7% |
| NSP4 | Genetically susceptible | ARR/VRQ | 0.2% | 0% | 2.6% |
| NSP5 | Genetically highly susceptible | ARQ/VRQ, ARH/VRQ, AHQ/VRQ, VRQ/ARQ | 0.1% | 1.3% | 44.6% |

[1]Classification system used in the UK for genetic resistance to classical scrapie and BSE [79].
[2]ARQ/ARQ may be scientifically reviewed.

atypical scrapie is explained in the last section of this paper. This susceptibility is given as the product of the elementary allelic factors [80]. Alleles ARR and AHQ are associated with resistance, whereas alleles ARQ, ARH and VRQ are associated with susceptibility [76]. However, several studies revealed that the resistance is not absolute: the scrapie agent can still infect a small proportion of these resistant animals (e.g. [81]). Moreover, one ARR/ARR sheep died after intracerebral inoculation of the BSE agent [82]. Results of an assessment of subclinical infection of sheep in a scrapie flock indicate that ARR or AHQ sheep are not healthy carriers of scrapie infection [44, 83] or at least were less infectious when comparing risk for lambs born to healthy dams either of resistant or susceptible genotype [44].

In goats, polymorphisms of the gene encoding the PrP have been found at ten codons [76] with codon 142 and codon 143 apparently linked to scrapie, whereas codon 154 and codon 222 appear to protect against scrapie [75, 84, 85]. Further information regarding genetic influences should be obtained through detection of new scrapie cases in goats (particularly after improvement of epidemiosurveillance networks) and additional research.

### *Propagation Pathway of Infectivity*

The precise route of transmission is unclear, but lambs exposed in the pasture show infectivity first in tonsils, retropharyngeal and mesenteric-portal lymph nodes, and intestine, which suggest infection via the alimentary tract, either in pre-natal period from scrapie agent in amniotic fluid or post-natal period from scrapie agent in a contaminant environment [86]. The susceptibility of sheep to oral infection depends notably on interactions between scrapie strain and host genotype [87]. The infectious dose probably also plays an important role [88]. Demonstration of infection depends on the sensitivity of the $PrP^{Sc}$ method used. The time of detection of $PrP^{Sc}$ infectivity depends on these two previous parameters.

Three phases can be discerned in the pathogenesis of natural scrapie (Figure 2): gut-associated lymphoid tissue (GALT) invasion; lymphatic dissemination; and neuroinvasion.

#### *GALT invasion*

After oral infection, primary sites where $PrP^{Sc}$ can be detected (as early as one month of age in natural scrapie infection in highly susceptible VRQ/VRQ sheep) and where perhaps replication of the scrapie agent first occurs are the GALT tissues of the oropharynx (such as palatine tonsils) and the gut (such as Peyer's patches) as well as the retropharyngeal and mesenteric lymph nodes [20, 87–89]. The surface epithelium above the GALT areas contains specialized cells, called M-cells. Pathogens can use M-cells to cross the mucosal barrier and gain access to the underlying lymphoid tissues [90], which could also be the case for the scrapie agent [87]. The earliest disease-specific $PrP^{Sc}$ accumulation seen was in tangible body macrophages within germinal centres. Later on it was detected in cells resembling follicular dendritic cells [91]. These cells might be carrying the scrapie agent from M-cells to the germinal centres of the underlying lymphoid follicles [89, 92, 93].

#### *Non-GALT dissemination*

When dendritic cells and macrophages gain access to the efferent lymph and subsequently the blood stream they might also produce the non-GALT dissemination that occurs to all other lymphoid tissues and notably the spleen and the lymph nodes, thereby entering the cortical

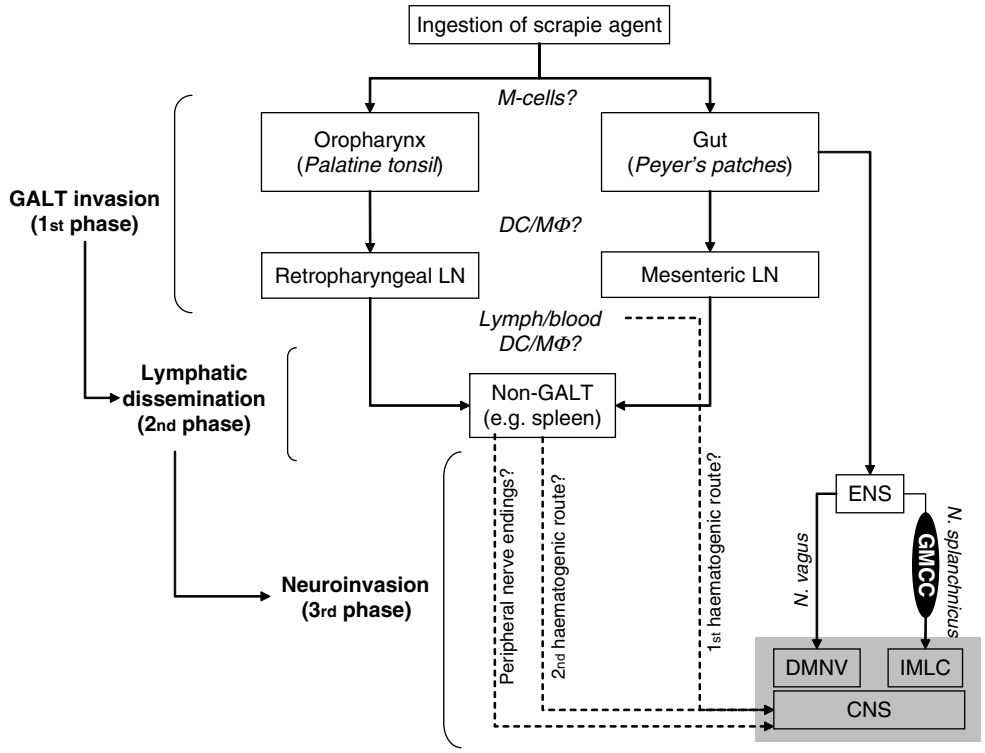


**Figure 2** Schematic model of the pathogenesis of natural scrapie in sheep [87]. Dotted lines: unproven hypothetical routes. DC: dendritic cell; MΦ: marcophages; LN: lymph node; GALT: gut-associated lymphoid tissues; ENS: enteric nervous system; CNS: central nervous system; DMNV: dorsal motor nucleus of the vagus; IMLC: intermediolateral column; GMCC: ganglion mesentericum craniale/coeliacum; M-cells: specialized cells that are contained in the surface epithelium above the GALT

and paracortical sinuses [87, 89]. In this preclinical stage, $PrP^{Sc}$ can be detected in biopsies of easily accessible lymphoid tissues (tonsils, third eyelid, rectal lymphoid tissues), thus enabling *ante mortem* tests for scrapie infection to be developed (e.g. [94, 95]). However, there have been reports of scrapie cases in which non-involvement of the lymphoid system was demonstrated [95, 96].

*Neuroinvasion*

In natural scrapie in sheep the nervous system is invaded about halfway through the incubation period [86]. The factors responsible for the neuroinvasion are not fully understood. However, it cannot be attributed to genotype alone [97].

Tissues and cellular localizations of $PrP^{Sc}$ indicate that $PrP^{Sc}$ was disseminated through different routes: through the enteric nervous system (proven route) and also probably through peripheral nerve endings, originating from infected non-GALT lymphoid tissues, via the lymph, and via the haematogenic route.

Transmission to the central nervous system (CNS) starts with invasion of the enteric nervous system of the gut, possibly facilitated by the infection of the Peyer's patches.

From there, the brain is invaded through an ascending infection along the parasympathetic and sympathetic efferent nerves innervating the gut. The portal of entry into the CNS is the dorsal motor nucleus of the *N. vagus*, which contains the parasympathetic neuronal cell bodies innervating the cervical, thoracic and abdominal viscera and is the intermediolateral column in the spinal cord, which contains the preganglionic neuronal cell bodies innervating the abdominal viscera and from where the scrapie agent eventually spreads to involve the entire neuraxis [87]. The sequential appearance of $PrP^{Sc}$ in the peripheral nervous system of the CNS, with satellite cells as early targets, indicates the periaxional transportation of positive cells in lymph nodes through supportive cells. $PrP^{Sc}$ positive cells in lymph node sinuses and in lymphatics indicate spreading by lymph. Focal areas of vascular amyloidal $PrP^{Sc}$ in the brain of some sheep indicate the haematogenous dissemination of $PrP^{Sc}$ [98].

Relative infectivity of tissues from sheep and goats with natural or experimental primary scrapie infections by oral route in function of type of organ is presented in Table 4.

## Transmission and Epidemiology

### *Transmission*

Although it is generally accepted that scrapie is an infectious, contagious disease, the precise transmission routes

**Table 4** Relative infectivity of tissues from sheep and goats naturally or primary experimental infected with scrapie by oral route in function of type of organ (adapted from [99])

| Tissues | Category | Infectivity[1] | $PrP^{Sc}$ |
|---|---|---|---|
| **CNS** | | | |
| Brain | IA | + | + |
| Spinal cord | IA | + | + |
| Retina | IA | NT | + |
| Optic nerve | IA | NT | + |
| Spinal ganglia | IA | NT | + |
| Trigeminal ganglia | IA | NT | + |
| Pituitary gland | IA | + | NT |
| Dura mater | IA | NT | NT |
| **Peripheral nervous system** | | | |
| Peripheral nerves | IB | + | + |
| Enteric plexuses | IB | NT | + |
| **Lymphoreticular tissues** | | | |
| Spleen | IB | + | + |
| Lymph nodes | IB | + | + |
| Tonsil | IB | + | + |
| Nictating membrane | IB | NT | + |
| Thymus | IB | + | NT |
| **Alimentary tract** | | | |
| Oesophagus | IB | NT | + |
| Fore-stomach | IB | NT | + |
| Stomach/abomasums | IB | NT | + |
| Duodenum | IB | NT | + |
| Jejunum | IB | NT | + |
| Ileum | IB | + | + |
| Appendix | IB | NA | NA |
| Large intestine | IB | + | + |
| **Reproductive tissues** | | | |
| Placenta | IB | + | + |
| Testis | IC | – | NT |
| Prostate/epididymis/ seminal vesicle | IC | – | NT |
| Semen | IC | NT | NT |
| Ovary | IC | – | NT |
| Non-gravide uterus | IC | – | NT |
| Placenta fluids | IC | NT | NT |
| Fetus | IC | – | – |
| Embryos | IC | ? | NT |
| **Musculo-skeletal tissues** | | | |
| Bone | IC | NT | NT |
| Heart/pericardium | IC | – | NT |
| Tendon | IC | NT | NT |
| **Other tissues** | | | |
| Lung | IB | – | – |
| Liver | IB | + | NT |
| Kidney | IB | – | – |
| Adrenal | IB | + | NT |
| Pancreas | IB | + | NT |
| Bone marrow | IB | + | NT |
| Skeletal muscle | IB | – | + |
| Tongue | IB | NT | + |
| Blood vessels | IB | NT | + |
| Nasal mucosa | IB | + | + |
| Salivary gland | IB | + | NT |
| Cornea | IB | NT | NT |
| Gingival tissue | IC | NT | NT |
| Dental pulp | IC | NT | NT |
| Trachea | IC | NT | NT |
| Skin | IC | – | NT |
| Adipose tissue | IC | NT | NT |
| Thyroid gland | IC | – | NT |
| Mammary gland/udder | IC | – | NT |
| **Body fluids, secretions and excretions** | IB | | |
| Cerebrospinal fluid | IB | + | NT |
| Blood | IB | + | ? |
| Cord blood | IC | NT | NT |
| Milk | IC | – | NT |
| Colostrum | IC | – | NT |
| Saliva | IC | – | NT |
| Sweat | IC | NT | NT |
| Tears | IC | NT | NT |
| Nasal mucus | IC | NT | NT |
| Bile | IC | NT | NT |
| Urine | IC | NT | NT |
| Feces | IC | – | NT |

IA, high-infectivity tissues: CNS tissues that attain a high titre of infectivity in the later stages of all TSEs, and certain tissues that are anatomically associated with the CNS; IB, lower-infectivity tissues: peripheral tissues that have tested positive for infectivity and/or $PrP^{TSE}$ in at least one form of TSE; IC, tissues with no detectable infectivity: tissues that have been examined for infectivity and/or $PrP^{TSE}$ with negative results; +: presence of infectivity or $PrP^{TSE}$; –: absence of detectable infectivity or $PrP^{TSE}$; NT: no tested; NA: not applicable; ?: controversial results; [1]: most bioassays of sheep and/or goat tissues have been conducted only in mice. In regard to sheep and goats, not all results are consistent for both species.

and their relative contribution to the overall transmission intensity remain poorly characterized [100] (Figure 4).

*Animal transmission*

The horizontal transmission of scrapie by contact between animals has been experimentally demonstrated (e.g. [60, 101]). However, all infected materials that play a role in this transmission are not known. Among them, placenta from infected ewes could transmit the disease to scrapie-free sheep and goats through the oral route [102]. $PrP^{Sc}$ has been found in the placenta and amniotic fluids, and oral uptake of placenta and amniotic fluids or materials contaminated by these is thought to play a major role in transmission between animals (e.g. [103–105]). Nevertheless, placental infectivity in such ewes is not systematic and $PrP^{Sc}$ accumulation in the placenta is controlled by polymorphisms of the foetal *PrP* gene. Moreover, $PrP^{Sc}$ is restricted mainly to placentome foetal trophoblastic cells [105]. Presence of scrapie infectivity in the placenta suggests the possibility of increased transmission of scrapie during the lambing season and results of modelling the spread of scrapie in a sheep flock provide strong support for this hypothesis [106]. Moreover, scrapie was more likely to occur in flocks that lambed in group pens or that always lambed in the same location [107].

The remarkable resistance of the scrapie agent to inactivation leads one to believe that it may survive in the environment for many years [4]. This idea is supported by

field experience showing that the scrapie agent can persist for at least 3 years in the environment [108] and from experiments showing residual infectivity in soil contaminated by scrapie hamster brain after 3 years internment [109]. More recently, it was found that TSE agent binds strongly to several minerals in the soil [110] and epidemiological investigations in Iceland show that the scrapie agent may persist in the environment for at least 16 years [111]. This long-term persistence of the scrapie agent in the environment favours the horizontal transmission of the disease, highlights the difficulties in eradicating scrapie and provides a serious warning of the risk of disposing sheep carcasses from scrapie-affected flocks by burial. However, a recent study indicates that composting may have a partial value as a means for degrading $PrP^{Sc}$ in carcasses and other wastes [112].

Maternal transmission could be defined as transmission of the infectious agent from infected ewes to their offspring *in utero*, during parturition, or in the immediate post-parturient period [4]. The last possibility is generally regarded as the most likely in practice [102].

Vertical transmission is defined as transmission of the infectious agent from an infected parent to offspring via germplasm at the time of fertilization or *in utero* during embryonic and foetal development [4] and is not currently demonstrated (e.g. [86]).

On several occasions, the spread of iatrogenic scrapie has been related to contaminated vaccines [3, 113, 114]. Direct transmission via other mechanisms such as hay mites has also been proposed (e.g. [115]) but no epidemiological evidence is known.

The basic reproductive number $R_0$, the average number of secondary infections produced by one infected individual introduced into a fully susceptible population, provides a quantitative assessment of the validity of an infectious agent to invade a susceptible host population. If $R_0$ is greater than unity, each infection on average more than replaces itself and the outbreak, at least initially, will escalate [116]. The within-flock reproductive number of scrapie was estimated between 1.5 and 6 (e.g. [100]).

*Flock transmission*

The main risk factors for introducing scrapie into flocks are purchase of female sheep from scrapie-affected flocks, sharing pastures with scrapie-affected flocks and sharing breeding rams [117]. This is in agreement with the view that the main routes for transmission of scrapie between flocks are movement of animals or animal-to-animal contact [18, 107, 118]. In France, purchase of proprietary concentrates was proposed to be a risk factor for scrapie, and the spread of the agent by contaminated meat-and-bone meal, analogous to the situation with BSE, was suspected [119]. More studies are necessary to consolidate this finding. Large farms and those with purebred sheep also appear to be at greater risk of having scrapie cases [107, 118].

## Diagnostics of Scrapie

### *Clinical Picture*

*Incubation period and age at the detection*

The incubation period, determined under experimental and iatrogenic conditions, was found to be long: around 18 to 23 months in sheep [3, 56, 57, 114], with the first clinical signs appearing in goats 2–3 months later than in sheep [3].

In the field, the incubation time is rarely known and only the age at detection can be estimated. Most scrapie-affected ewes and goats are two to eight years old (Figure 3) [3] with the majority of cases occurring between two and five years of age [3, 5, 120, 121]. The annual scrapie incidence is roughly equal for ewes between one and two years old and ewes two to three years old [44]. Some scrapie cases are above seven years [1, 3, 55], some below one year [6, 26, 122, 123]. The youngest scrapie-affected sheep was a six-month-old lamb in France [6]. In an endemic situation, the age at detection decreases in function of successive generations of scrapie cases. This observation is probably closely linked with an increase in scrapie exposure [40, 37, 124]. For a particular country the age at detection varies also in function of breed, host genotype [44, 125] and the use of active surveillance (infected animals can be identified before the onset of clinical signs) [17].

*Clinical scrapie*

Animals of both sexes are affected [88]. Usually, only one or a few sheep are affected simultaneously in a flock (http://www.defra.gov.uk/corporate/vla/science/documents/science-scrapie-res.pdf).

Owing to the damage to the CNS, affected animals will usually show neurological disorders that can be classified in four groups: behavioural changes, locomotor incoordination (ataxia), pruritus and abnormal movements (tremor, especially of head and neck), which progress to recumbency and death. General signs are limited to weight loss. As a consequence of the variety of signs, several names are attributed to the disease, basically in function of the country: *scrapie* (English, pruritus causing scraping or rubbing against fixed objects), *tremblante* (French, trembling), traberkrankheit (German, trotting disease causing ataxia) or *rida* (Icelandic, weight loss).

The duration of the clinical disease, from the earliest clinical signs until death or slaughter, may be under two weeks or as long as one year [4, 121]. The average length is about one to six months [4, 126]. This duration depends, above all, on the abilities of the flock attendant to recognize any clinically suspect signs [18, 127]. Of confirmed scrapie cases, 16% were found dead without exhibiting signs before death [121].

The onset of clinical signs often starts with a slight change in behaviour. The animals become more nervous or aggressive and may separate themselves from the rest

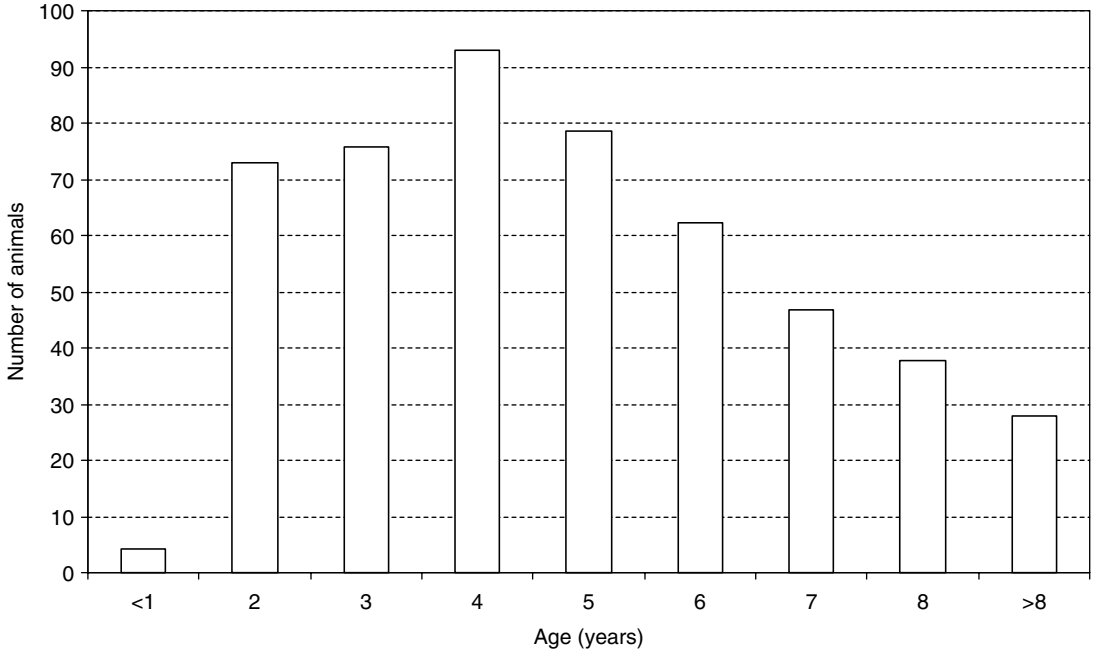


**Figure 3** Age at death from scrapie in sheep and goats [3]

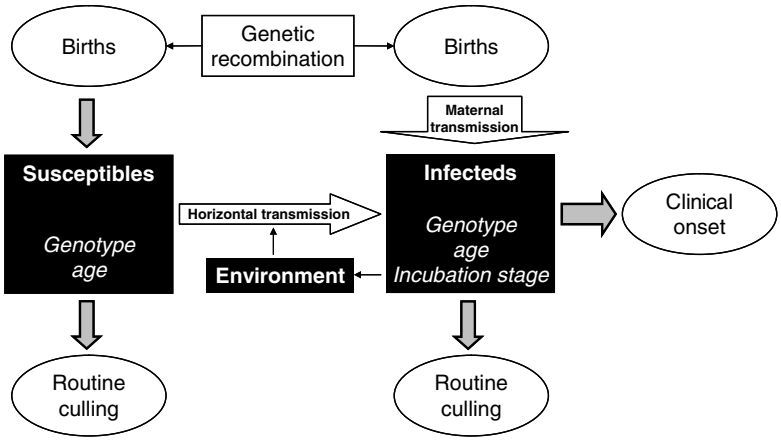


**Figure 4** Proposed within-flock scrapie transmission model [189]

of the flock, especially when at pasture and some fail to return when the flock is gathered [4, 88]. Abnormal postures can also be also observed (e.g. pressing the head).

Locomotor incoordination is characterized by a staggering walk. Motor incoordination includes a trotting gait of the forelimbs and a galloping movement of the back legs. This gait is especially noticeable when the animal is made to get up or to run, with the animal possibly stumbling over obstacles or falling. As the disease progresses, there may be severe ataxia of the hind limbs causing the animal to sway, support its hindquarters against a fence when standing and having difficulty rising [4, 88]. Finally, the affected animal shows tetraparesis.

Scrapie acquired its name from the feature of sheep rubbing themselves against fixed objects. Pruritus may be subtle and go undetected or so dramatic that an animal will rub off most of its wool [18]. Some sheep may exhibit a nibbling reflex or grinding teeth when rubbing themselves or when scratched by a hand over the lumbar area of the back.

Tremors, especially of the head and neck, are often late and of lower intensity. Other abnormal movements such as abnormal head carriage (lolling, nodding or shaking of the head) may be present. Hypersensitivity may be observed: an affected animal may appear normal if left undisturbed, but when handled, tremors may become excessive and the animal may even fall in a convulsion-like

state (e.g. milk ewes cannot support milk sleeves and fall) [4, 88].

In goats and mouflon, clinical signs observed were similar to those described for domesticated sheep [2] but reports of the clinical signs in the latter species are limited and must be taken with caution [3, 18, 128]. Other clinical signs may be also observed in goats: aggressiveness, difficulty in milking (e.g. resistance to milking by kicking of the hind limbs), cannibalism, premature kidding and pica [3, 126].

*Clinical considerations*

It is essential to emphasize that not all animals exhibit the full range of clinical signs of the disease. There can sometimes be extreme variation in the clinical signs of individual animals in function of the genotype (and thus indirectly of the breed). An animal showing severe pruritus may show little if any incoordination (*scratching* form) and vice versa [18].

Despite considerable educational efforts, confusion of owners still remains regarding the clinical picture of scrapie [129, 130] and under-reporting of cases is frequent [131]. Moreover, inconsistencies in available information, misclassification of sheep with clinical suspected scrapie and changes in the national scrapie control and eradication programmes induce biases in some clinical studies [132]. In order to improve passive scrapie surveillance, it is necessary to use a standardized clinical evaluation record form indicating presence or absence of all possible clinical signs linked with TSEs in ruminants. These clinical signs should include behavioural change, sensory problems, locomotory problems, posture anomalies, and general problems. In analogy with BSE [133], decision support tools may be developed to perform a more appropriate passive (continuous) epidemiosurveillance in case of scrapie (e.g. [134]). The possibility that, in larger flocks, sheep showing signs are not noticed, even if the shepherd knows the clinical picture, cannot be excluded.

Any clinical sign is a pathognomic clinical sign [135]. Only the simultaneous presence of several clinical signs should be called a clinical suspicion of scrapie (Table 5) and this suspicion should always be confirmed by laboratory diagnostic tests [134].

*Differential diagnoses*

Diseases that must be taken into account in the differential diagnosis of scrapie are skin diseases caused by ectoparasites (e.g. lice and mites), acute neurological disorders (e.g. listeriosis, Aujeszky's disease, rabies, pregnancy toxaemia, plant or chemical toxins and pasture tetany) and chronic neurological disorders (e.g. abscess at the base of the brain, cenurosis, maedi-visna, peripheric neuropathies) [4, 136]. More detailed lists of frequently reported neurologically expressed disorders in sheep and goats are available in the literature (e.g. [5, 11]).

**Table 5** Main critical scrapie clinical signs [134]

| Sign description[1] |
|---|
| Abnormal head position |
| Blindness or visual impairment |
| Change of temperament |
| Confusion or disorientation |
| Fleece abnormalities |
| Licking and excess salivation |
| Locomotion problems or changes |
| Nibbling reflex (when back scratched) |
| Nibbling biting |
| Poor condition |
| Pruritus and scratching |
| Staring or depression |
| Trembling/shaking/twitching |

[1]Fourteen groups of signs are the standard output from the Scrapie Notifications Database in Great Britain.

Since the publication of the EFSA opinion (2003) [137], the European Commission had sponsored large-scale surveillance for TSEs in small ruminants (Table 6) and, since 2005, each positive index case detected in a TSE-affected flock was required to be further analysed using newly-developed tests capable of *in vitro* discrimination of BSE and scrapie in sheep. Currently the BSE must be also considered in the differential diagnosis.

## *Rapid Tests*

The active TSE surveillance in small ruminants is based on post-mortem rapid tests. Biochemical methods (mainly western blot and ELISA test) are performed on unfixed tissue homogenates. Addition of PK permits total and partial hydrolysis of $PrP^{C}$ and $PrP^{Sc}$, respectively. The resistant PrP ($PrP^{Res}$) is demonstrated by means of different specific antibodies. Several stages of purification or concentration are required in order to enhance the performance of the tests and their speed of execution.

Using the western blot, $PrP^{Sc}$ appears in the form of three bands (non-glycosylated, mono-glycosylated and bi-glycosylated), which constitute the electrophoretic profile. Results are obtained within 7–8 h [139], whereas ELISA tests yield results after 4 h [140]. The latter method is better suited for the analysis of a large number of samples.

Initially, in the absence of rapid TSE tests evaluated on material from small ruminants, all the rapid tests, approved for the detection of BSE in cattle, were accepted for testing small ruminants. The list of these rapid tests in Annex X to Regulation (EC) No. 999/2001, as amended by regulation (EC) No. 1053/2003, includes five tests: Prionics Check Western, Enfer, Bio-Rad TeSeS, Prioniscs Check LIA and InPro CDI-5. Later, in a first evaluation, five tests have been evaluated and approved on brain material from sheep: Bio-Rad TeSeE, Bio-Rad TeSeE Sheep/Goat, Enfer TSE, Pourquier's-LIA Scrapie

**Table 6** Results of the epidemiosurveillance of TSEs in sheep and goats in function of exit stream (European Union, Year 2005) [17, 138]

| Country | Sheep population | Goat population | Culled for destruction | | Not slaughtered for human consumption | | Slaughtered for human consumption | | TSE suspects | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Nt | Np | Nt | Np | Nt | Np | Tested | Positive |
| Austria | 325 495 | 54 607 | 0 | 0 | 5222 | 0 | 272 | 0 | 2 | 0 |
| Belgium | 146 030 | 26 237 | 8 | 0 | 1621 | 2 | 703 | 0 | 53 | 0 |
| Cyprus | 264 552 | 407 917 | 0 | 0 | 103 | 30 | 3808 | 123 | 2813 | 726 |
| Czech Republic | 104 000 | 13 000 | 0 | 0 | 536 | 1 | 75 | 0 | 53 | 0 |
| Denmark | 105 000 | 10 000 | 0 | 0 | 5193 | 0 | 346 | 0 | 5 | 0 |
| Estonia | 31 500 | 4300 | 0 | 0 | 287 | 0 | 981 | 0 | 0 | 0 |
| Spain | 23 485 947 | 3 162 056 | 5106 | 78 | 22 731 | 20 | 41 856 | 18 | 40 | 8 |
| France | 8 962 467 | 1 228 764 | 10 753 | 249 | 70 909 | 55 | 112 801 | 13 | 47 | 26 |
| Germany | 2 078 500 | 160 000 | 3769 | 18 | 32 619 | 18 | 16 451 | 8 | 66 | 0 |
| Greece | 9 042 000 | 5 362 000 | 269 | 9 | 2513 | 43 | 7911 | 15 | 425 | 29 |
| Ireland | 4 850 100 | 9000 | 1670 | 24 | 10 453 | 21 | 10 689 | 1 | 6 | 5 |
| Italy | 7 951 640 | 961 028 | 5544 | 281 | 11 096 | 20 | 39 474 | 15 | 36 | 18 |
| Latvia | 39 200 | 15 000 | 0 | 0 | 74 | 0 | 9 | 0 | 0 | 0 |
| Lithuania | 16 900 | 27 200 | 0 | 0 | 87 | 0 | 947 | 0 | 0 | 0 |
| Luxemburg | 7442 | 2400 | 0 | 0 | 481 | 0 | 396 | 0 | 0 | 0 |
| Hungary | 1 296 000 | 82 000 | 0 | 0 | 6105 | 0 | 3187 | 0 | 44 | 0 |
| Malta | 14 861 | 5374 | 0 | 0 | 279 | 0 | 24 | 0 | 18 | 0 |
| The Netherlands | 1 276 000 | 274 000 | 1018 | 27 | 11092 | 23 | 28 063 | 14 | 2 | 0 |
| Poland | 331 283 | 192 470 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Portugal | 3 355 616 | 501 857 | 0 | 0 | 22 907 | 25 | 55 244 | 32 | 3 | 0 |
| Slovenia | 105 660 | 23 291 | 421 | 101 | 2111 | 4 | 233 | 0 | 11 | 0 |
| Slovakia | 325 521 | 39 225 | 8 | 0 | 2461 | 7 | 258 | 2 | 1 | 0 |
| Finland | 67 400 | 4800 | 280 | 2 | 1121 | 3 | 762 | 0 | 4 | 0 |
| Sweden | 449 000 | 5500 | 33 | 0 | 3409 | 1 | 97 | 0 | 0 | 0 |
| UK | 24 574 660 | 88 453 | 104 | 4 | 11 091 | 37 | 15 098 | 30 | 355 | 179 |
| EU-25 | 89 206 774 | 12 660 479 | 28 985 | 793 | 224 501 | 310 | 337 684 | 271 | 3984 | 993 |
| % of positive | | | | 2.74 | | 0.14 | | 0.08 | | 24.92 |

Nt: number of tested animals; Np: number of positive animals.

and Prionics Check LIA Small Ruminants. In a second evaluation, two additional tests were approved: IDEXX HerdChek BSE-Scrapie antigen and the InPro CDI-5 test [141, 142].

### *Confirmatory Diagnosis*

All samples with a positive result in one of the rapid tests must be retested in the national reference laboratory using one of the OIE-approved confirmatory methods [143]. These are histopathology, immunohistochemistry (IHC), electron microscopy and scrapie-associated fibrils (SAF) immunoblots. For practical reasons, mainly the IHC and SAF immunoblots are of relevance today [46].

*Histopathology*

The histological changes are typical: microscopic lesions in the CNS consist of bilaterally symmetrical, non-inflammatory neuronal vacuolation, neuronal degeneration and loss, vacuolation of grey matter neuropil, astrocytosis and (sometimes) occurrence of amyloidal plaques. These findings are most prominent in the medulla, pons, midbrain and thalamus. It should be noted that brain tissue from apparently normal sheep may display the occasional vacuolated neuron [4]. Histopathological changes occur late during the infection process. The samples must be of high quality and without autolysis [88]. The histopathological differentiation between classical and atypical scrapie is described in the section entitled 'Atypical scrapie cases'.

*IHC*

Immunohistochemical staining is performed on formalin-fixed, paraffin-embedded sections of suspected animal tissues and submitted to pre-treatment (e.g. formic acid, low action of proteases, and hydrated autoclaving) with the aim to inhibit the immuno-reactivity of $PrP^{C}$ (increase of specificity) and to increase the immuno-reactivity of $PrP^{Sc}$ (increase of sensitivity) [144, 145]. The reactivity of $PrP^{Sc}$ is then revealed through the use of different antibodies (e.g. [146–148]). Advantages of this technique are the capacity to reveal a few accumulations of $PrP^{Sc}$ and the possibility of working on altered tissues or after post-mortem autolysis [149]. Moreover, IHC on lymphoid tissues (tonsil, third eyelid) permits also subclinical detection of ovine scrapie (e.g. [87, 140, 150]). Correlation of the performance of immunoblot and IHC methods is generally in agreement, but it is obvious that the neuroanatomic sites of examination are critical, certainly in preclinically, naturally affected animals [97, 139].

*SAF*

Abnormal $PrP^{Sc}$ can also be demonstrated in unfixed brain extracts in the form of SAF visualized by negative stain electron microscopy [151–153]. This technique may be useful when available brain tissue is unsuitable for histological examination because of post-mortem autolysis [154]. Modifications of this technique have also permitted recognition of SAF in fixed tissues [155]. Recently, SAF immunoblotting techniques have also been described to permit handling of information by means of a camera (e.g. [46]).

*Strain typing*

Numerous distinct TSE strains have been identified in bioassays by serial passages in mice of the scrapie agent from a host of sheep and goats [64]. The most useful characteristics to distinguish these strains are the length of the incubation period between initial infection and the development of clinical disease, and the type of pathological changes that are seen in the brains of infected animals [156–158]. TSE strains have also been found to differ in their clinical manifestations, their ease of transmission to new species and their susceptibility to inactivation by heat and chemicals [64]. In contrast to $PrP^{C}$, $PrP^{Sc}$ exhibit a remarkable resistance to PK, with, however, PK cleavage of the N-terminal of $PrP^{Sc}$ being more or less efficient, depending on the particular prion strain [159–162]. Size differences of PK-treated, non-glycosylated $PrP^{Sc}$ can be shown by high-resolution SDS-PAGE (polyacrylamide-gel electrophoresis) and immunoblotting or by using monoclonal antibodies binding to an epitope located on the ragged end of PK-cleaved $PrP^{Sc}$. Recently, a novel biochemical BSE/scrapie strain typing strategy in sheep was described (TSE discriminatory testing termed FLI-test), which includes determination of the molecular masses, antibody binding affinities and glycosylation pattern of the TSE-induced abnormal prion protein [46]. These biochemical techniques complement the traditional strain typing techniques of bioassaying the isolates in defined conventional and, more recently, transgenic mouse lines [46].

It is now established that these methods can also be used to type the TSE strain present in a naturally infected host (e.g. [46, 162]). However, it is still not clear what the basis of TSE strain variation is at the molecular level [64].

*Discriminatory testing for BSE*

Scrapie and BSE in sheep or goats can only be differentiated at present on the basis of post-mortem testing. This discriminatory testing for BSE is based on IHC, ELISA and/or western blotting of brain (or lymphoid tissue) for abnormal prion protein in animals experimentally challenged with infectious material taken from cases of cattle BSE defined as 'typical' or consistent with the original clinical and histopathological case definition of the diseases [163, 164]. The methodologies have been evaluated, and shown to be fit for purpose, in an European Union-wide ring trial using a variety of test materials covering oral and intracerebral challenged sheep of a limited range of PrP genotypes, and include cases of secondary intracerebral transmission of sheep BSE to sheep [165]. However, the sample set was necessarily restricted to

**Table 7** Preventive and control measures against scrapie applied in the world [18, 170]

| |
|---|
| **Preventive measures** |
| Maintenance of closed flock, especially with regard to pregnant ewes (biosecurity rules)<br>• any replacement ewes or breeding rams should originate from flocks not known to be affected with scrapie and<br>• have management practices precluding the introduction of scrapie |
| The use of selective breeding programmes based on *PrP* genotype |
| **Control measures** |
| Educating producers, veterinarians and others about clinical signs of scrapie |
| Identification of sheep and goats in commerce to allow for effective tracing of scrapie-positive and exposed animals |
| Finding infected and source flocks through the testing of exposed animals (out of known infected flocks, through slaughter and fallen stock surveillance) |
| Complete flock depopulation of infected and source flocks |
| Depopulation of certain genetically related animals<br>• based on maternal transmission or<br>• based on genotype susceptibility |
| Depopulation of at risk animals based on suspected pathways for transmission, such as lambing groups |
| Depopulation of affected animals only |
| Cleaning and disinfection of premises |
| A period during which susceptible species may not be restocked on the premises |

what was available and no meaningful estimate of the sensitivity and specificity of these tests has been made. Nor were these tests evaluated for their ability to discriminate types of BSE (cattle TSE) now regarded, on molecular and histopathological criteria, to lie outside the original case definition of BSE [166–168]. These forms of cattle TSE have been dubbed H-type BSE (H; $PrP^{Sc}$ fragment of higher molecular weight) and L-type BSE (L; $PrP^{Sc}$ fragment of lower molecular weight or BASE). The significance, origin and transmissibility of these H- and L-types of BSE to sheep are only speculative at present.

*Other tests*

Recent tools such as protein misfolding cyclic amplification (PMCA), which combine the use of crude brain homogenate in a reaction tube with that of sonication and iterative cycles of *in vitro* conversion, may be more suitable for demonstrating new infectivity arising from the conversion of $PrP^{C}$ into $PrP^{Sc}$ [169].

## Prevention and Control of Scrapie

In the absence of an effective treatment or vaccine for scrapie, a range of measures should be applied to prevent or control the disease in the world (Table 7).

Reporting of clinical suspicions in small ruminants is a useful method to detect cases of scrapie, but, as shown in Germany, sometimes very few clinical cases are reported [171]. Reporting can be improved by training producers, veterinarians and others involved about clinical signs of scrapie.

Identifying the risk factors associated with the introduction and the spread of scrapie within and between flocks is of prime importance (see the section on transmission and epidemiology). These risk factors are identified by direct transmission and pathogenesis research but also by epidemiological studies and modelling.

Control measures require a correct identification of flocks and small ruminants, depending on the species and sanitary status of the herds. In infected sheep flocks, the strategy should currently be selective depopulation of animals showing clinical signs of scrapie and genetically susceptible and/or related animals, whereas in healthy flocks (particularly in breeding flocks) the strategy is based on biosecurity and the use of selective breeding programmes based on *PrP* genotype [88].

In goats, the control of scrapie is based on depopulation of animals in infected flocks because of the absence of known genetic susceptibility to scrapie [88].

## Atypical Scrapie Cases

Classical cases of scrapie – the so-called 'typical' scrapie – are associated with vacuolization, the accumulation of a relatively protease-resistant form of abnormal prion protein consistently in the brain stem at the level of the obex and are usually, but not uniquely, found in animals carrying an ARQ or VRQ PrP allele.

In 1998, a new TSE strain, designated scrapie Nor98, was found in Norwegian sheep [172]. Active surveillance of TSE in small ruminants, implemented since 2002 in the European Union, has allowed the quick identification of a growing number of cases associated with this unusual strain [173], mainly in sheep but also in goats in several other European countries (e.g. [17, 174–181]). These 'atypical' isolates were characterized by discrepancies in results obtained by different rapid diagnostic tests based on $PrP^{Sc}$ detection (missed by some of the usual rapid tests), by difficulties in confirmation by OIE-recommended diagnostic methods (their identification

requires very sensitive methods), by a different distribution of pathological changes and of $PrP^{Sc}$ in the CNS (more abundant in the cerebellum and in the cortex than in the brain stem, in contrast with classical scrapie), and by the PrP genotypes affected most frequently and by older age of the case animals at the time of detection [168, 175, 182, 183]. These Nor98 cases, the prototypes of 'atypical' TSE, have little or no vacuolization or abnormal PrP at the obex, but in most cases exhibit an intense cerebellar $PrP^{Sc}$ deposition characterized at a molecular level by a smaller and less stable protease-resistant core of $PrP^{Sc}$. Nor98 and other 'atypical' cases subsequently identified are more often but not uniquely found in animals carrying alleles not usually associated with classical scrapie. For Nor98, the genotype correlation has been shown to implicate another codon, L141F [182]. The differentiation between classical and atypical TSE cases in small ruminants has been extensively documented in an EFSA opinion [176].

Overall results of biochemical studies suggested a lower protease resistance of the $PrP^{Sc}$ form that might explain such discrepancies between diagnosis tests [168]. This $PrP^{Sc}$ fragment was recently found to be cleaved at both N- and C-terminal ends of the protein, in contrast with only the N-terminal cleavage found in classical scrapie and BSE [184].

The risk for atypical scrapie was highest in sheep carrying phenylalanine (F) at position 141 ($AF_{141}RQ$) and/or the AHQ haplotype. However, atypical scrapie also occurred with a notable frequency in sheep with the PrP haplotypes ARR and/or ARQ in combination with leucine at position 141 ($AL_{141}RQ$). Furthermore, some atypical scrapie-positive sheep carried the PrP genotype ARR/ARR (known to be more resistant to the classical scrapie) [171, 183].

Several significant epidemiological differences were also observed between classical and atypical scrapie cases with regard to the number of scrapie-affected sheep within a flock (fewer animals in the case of atypical scrapie, $n>1$ in >50% of classical infected flocks, and $n=1$ in >90% of atypical infected flocks) and the age distribution of the scrapie-positive sheep (older animals in the case of atypical scrapie) [171].

In addition, the epidemiology of scrapie Nor98 differs from the classical scrapie in that no risk factors that indicate transmission of scrapie Nor98 between flocks by movement or direct contact between animals were found [173].

An overview and updated information on atypical cases including the indication of criteria for the definition of atypical cases or to determine certain groups of atypical cases is given in an EFSA opinion (2005) [176].

Uncertainties remain regarding the pathogenesis of the atypical scrapie, as well as regarding its potential transmissibility within the affected species and to other species [168]. It is possible that some of these newly recognized forms of TSE are similar to 'sporadic' forms of neurodegenerative diseases, including most of the cases of CJD in humans [64] and, in all these situations, the occurrence of such 'sporadic' cases would raise the question of the origin of such diseases [168].

## BSE in Sheep

Thus far, no BSE cases have been confirmed in sheep under natural conditions despite the fact that more than 2000 sheep brains affected with scrapie were analysed with a test designed to distinguish scrapie from BSE [165]; but two historical cases of BSE in goats born in the 1990s have been identified meanwhile: one pre-clinical (French case) [185] and one clinical (UK) [186]. In addition, after oral experimental infection of BSE agent (5 g of BSE cattle brain inoculum) in susceptible sheep (ARQ/ARQ genotype), it was confirmed that BSE can transmit either *in utero* or via perinatal close sheep contact. However, the transmission would be limited to one family line [187]. Moreover, other experimental infection with a lower dose and the use of less susceptible genotypes failed to demonstrate this transmission [188]. However, it was recently demonstrated that the BSE agent, after intracerebral challenge, can infect sheep believed to be the most resistant genetically to prion diseases (ARR/ARR genotype) and in the absence of clinical signs [190]. This suggests that sheep may be silent carriers of the BSE agent [188]. Possible transmission of the BSE agent to ovine species under natural conditions remains still on debate.

## Conclusion

Currently, the aetiology of scrapie is considered an infectious disease with a maternal and horizontal contagious transmission, where host genetic factors play a central role. Uncertainties remain regarding the pathogenesis of some numbers of a new TSE form in small ruminants, as well as regarding their potential transmissibility within the affected species and to other species. The development of prevention and control programmes should be assisted by new science findings.

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