Introduction

Written documentation regarding scrapie dates back to the 18th Century. Despite ongoing research over the years, this insidious neurological disease of sheep and goats still poses more questions than provides answers. The traits and characteristics of the disease have led many to believe that finding solutions for its demise would not be difficult, yet scrapie has usually evaded elimination. Strategies for control and eradication have been varied. In countries where scrapie is endemic, schemes have been modified based on the latest science; they have been finely tuned or totally overhauled, still success has not yet been achieved. Words from a 1759 German scientist still bode a valid warning, as follows:

‘The best solution for the shepherd who notices that one of his animals is suffering from scrapie is to dispose of it quickly... A shepherd must isolate such an animal from healthy stock immediately because it is infectious and can cause serious harm to the flock’ (87).

The common characteristics of the transmissible spongiform encephalopathy (TSE) family render scrapie elusive in regard to eradication and control. Experience shows that prevention is a priority in countries, regions or flocks where the disease is not present. Rapid elimination of the disease immediately after introduction is the essential strategy if prevention measures do not succeed. Failure to prevent or eliminate in an expedient manner allows the silent spread of the disease during the prolonged incubation period (months to years). For those countries or regions in which the disease has become endemic, elimination efforts have spanned decades and in most cases have not been successful (27). The traits of the disease have been the primary cause of this lack of success. The emergence of bovine spongiform encephalopathy (BSE) in the world has sparked much more research which in turn has provided information to facilitate control and eradication efforts. Knowledge gained over the last twenty years has provided an expanded repertoire of diagnostic techniques, as well as information on genetic influences and transmission. While BSE has precipitated the research for tools to eliminate scrapie, it has also spurred efforts in the world to eradicate scrapie.

Despite the new information provided by research to date, there is still a vast void in the knowledge of scrapie, as follows:

a) the aetiological agent of the disease has still not been fully characterised

b) the pathogenesis of scrapie is not fully understood; a better understanding may help to target tissues for diagnostic purposes, to block the progression of or to prevent the disease

c) all routes of transmission and their relative importance are not known; although more information has been gained about the role of the placenta and the neonatal period, other routes of transmission need to be studied to prevent the spread of the disease (e.g. What is the risk from nasal discharge, urine and pasture contamination? Or, could there ever be a blood-borne risk such as through the re-use of needles during routine procedures like vaccination or mechanical transmission via blood-sucking insects?)
d) while much information has been gained in the area of host genetic factors influencing scrapie, significant information need to be obtained before one can be sure that genetics will provide all the answers for elimination of the disease; for example:

- are the genotypes that do not display evidence of clinical disease infected with the scrapie agent and do they pose a risk for other sheep?
- will the agent adapt to the more resistant genotypes and become a problem in the future?
- are there genes, such as Doppel (94), or others as yet unidentified, that modify or supplement the effects of the host prion protein (PrP) gene in regard to scrapie?

e) advances in diagnostic tests have allowed for disease confirmation in autolysed or frozen brain tissue and some tests have also provided the first steps as a preclinical means of diagnosing scrapie in the live animal (101, 102, 148); however, these techniques either do not allow detection at the earliest possible time post infection or provide an extreme logistical challenge

f) what methods of disposal fully inactivate the agent?

g) is scrapie the source of BSE?

h) are scrapie and chronic wasting disease the only TSEs with cycles in nature?

Current information will have to be supplemented if the world is to succeed with a plan for elimination.

Geographical distribution

Scrapie has been reported world-wide and affects many sheep-producing regions. Substantiating the exceptions is a major challenge. In the past, many countries relied on a passive surveillance system to determine the presence or absence of scrapie. Lessons learned from BSE dispel the notion that making a disease reportable is enough to determine ‘scrapie-free status’. The lack of a preclinical screening test which definitively detects all scrapie infection adds to the complexity of ascertaining freedom from the disease. It is rarely if at all possible to justify freedom from scrapie on the basis of passive surveillance alone, and since many outside factors influence reporting, actual prevalence continues to remain obscure in most countries. This is illustrated by the recent discovery of scrapie in Finland (97) by way of the European Union (EU) mandatory statistical sampling of at-risk (fallen stock and emergency slaughter) sheep and goats and slaughter surveillance of healthy adult animals. Another means of active surveillance would be to target sampling at those breeds or type of animals which may have been imported or have been derived from imports of scrapie-affected countries.

Due to the nature of the disease it is difficult to develop all inclusive guidelines which would establish scrapie-free status for a country. Questions arise regarding how much and for how long is surveillance needed to determine scrapie-free status. Currently, the Office International des Epizooties (World organisation for animal health) is establishing criteria to use for the establishment of country-level scrapie-free status.

Active surveillance may also be useful in determining a more accurate prevalence level in countries where the disease is known to be endemic. For example, in the United States of America (USA), a slaughter surveillance project along with mandatory identification was designed to provide information on national and regional prevalence. Active surveillance can also be used as a tool to evaluate the success of a control/eradication programme.

Historical evidence and spread of scrapie

As cited earlier, the first reports of the existence of scrapie appear in 18th and 19th Century literature from England and Germany. The earliest definite record of the occurrence of scrapie was in Great Britain in 1732 (89).

Throughout the 1700s and 1800s, scrapie was reported in many breeds of sheep in England as well as in continental Europe. Spread of the disease was reported to have been through the importation of certain breeds of sheep, such as the Merino (107).

Throughout the 1900s, scrapie is thought to have spread across the world especially post World War II, through the movement of sheep incubating the disease. One illustration of this (see below) is the export of British Suffolks or Hampshire Downs throughout the world and the subsequent diagnosis of scrapie in these sheep or their contacts (10, 17, 25, 107, 117, 137, 146) (Fig. 1).

This documentation of disease spread through the movement of preclinical scrapie-infected sheep illustrates the need for sound preventive measures which include restrictive import requirements and if possible preclinical testing. If scrapie were to be introduced, rapid detection and elimination are essential. The above documentation also demonstrates that post-entry requirements play a major role in preventing the establishment of scrapie if introduced. For example, by having post-entry restrictions and requiring complete depopulation of imports and contact sheep after scrapie was detected, Australia and New Zealand appear to have successfully eliminated the disease after introduction through imported animals. This is unlike the situation in Canada and the USA where scrapie became endemic.

In recent years, many countries have continued to report the absence of scrapie. However, no details on surveillance strategies are available. It may be anticipated that in the future, some form of active scrapie surveillance, much like that which exists for BSE, may be required to document scrapie-free status.
It is also likely that new diagnostic techniques will be available to assist with such surveillance.

**Scrapie in goats**

Naturally occurring scrapie in goats has been reported in Canada (131), Cyprus (138, 139), Finland (97), France (22), Greece (86), Italy (18), Switzerland (38), the United Kingdom (UK) (3, 11, 58) and the USA (65, 145).

**Pathogenesis**

Although the pathogenesis of scrapie is still not fully understood, increasing knowledge regarding disease progression is essential if prevention and eradication of scrapie are to be achieved. For example, recent research has assisted in targeting additional tissues for diagnostic purposes, especially in the preclinical phase of the disease. Studies in progress or planned for the future may help to provide the knowledge required to block progression or prevent the occurrence of the disease. Perhaps most importantly, knowledge of pathogenesis provides information on the potential for secretion or excretion of the agent, providing the avenue for exposure of healthy animals. In addition, scrapie has been transmitted iatrogenically to other sheep and goats through the use of certain biological products made from scrapie-infected tissues. Knowledge of tissues which may harbour infectivity allows safer sourcing.

Studies to date indicate that there is no evidence that scrapie is a human health risk (13, 59, 79).

However, since BSE in sheep appears to have a similar pathogenesis as scrapie, if BSE in sheep were to exist naturally, further information would be essential for public health reasons.

Early scrapie research in mice, sheep and goats suggested an oral route of infection and early replication/propagation of the agent in lymphoreticular tissues which include tonsil, spleen, retropharyngeal and mesenteric lymph nodes as well as peripheral nervous tissue. Over time the agent spreads to most lymph nodes with evidence that it invades a number of other non-neural tissues. Usually, replication/propagation continues in the lymphoreticular system (LRS) for months before evidence of infectivity can be detected in the brain (34, 51, 52, 53, 54).

Recent studies have repeatedly confirmed these findings, although most of the work regarding disease progression has been performed using techniques to detect the partially protease form of the prion protein (PrPSc: scrapie-associated prion protein) in tissues rather than confirming absolute infectivity (1, 2, 60, 76, 77, 148, 149). The detection of PrPSc can be accomplished with greater ease and speed than the
Detection of infectivity. However, it must be noted that although there have been good correlations between the presence of PrP Sc and infectivity (114), TSE infections have been reported in the absence of detectable PrP Sc (85, 91, 115, 127). In addition, it appears that certain sheep (perhaps due to a genetic influence) may become infected with scrapie and progress to clinical disease without having detectable PrP Sc (1, 102, 115, 148) or infectivity (52) in the LRS. In addition, PrP Sc in urine has been reported in the absence of detectable infectivity (122).

Detection of PrP Sc in the LRS has been recorded in Romanov sheep with natural scrapie at as early as two months of age (1) and in Texel and Swifter sheep at four months of age (121). Interestingly, PrP Sc was not detected in the LRS of Suffolk sheep with natural scrapie, until eight months of age (76). This is consistent with the first detection of infectivity in the LRS of Suffolk sheep at ten months of age (54).

The use of immunohistochemistry (IHC) to detect PrP Sc has been extremely useful in providing additional information at a cellular level. Generally, PrP Sc has been found mainly in primary and secondary B follicles of the LRS. The immunolabelling appears as a reticular pattern in the centre of the follicle, indicating deposits in follicular dendritic cells. There is also staining in macrophages (1, 76, 77, 148). In the central nervous system (CNS), PrP Sc accumulates primarily in glial cells (astrocytes) and neurons (1, 147). A thorough review of the immunobiology of the TSEs is provided by Mabbot and Bruce (88).

The means by which the agent moves from the LRS to the brain has not fully been established. Blood and nerve fibres have both been incriminated. Data in several papers have suggested that infection occurs through LRS tissue followed by replication in these tissues, with spread to the CNS through the autonomic nervous system (149). In highly susceptible sheep, PrP Sc was detected in the enteric nervous system spanning from the oesophagus to the rectum. In sheep thought to have a lower genetic susceptibility, PrP Sc was found in the fore-stomachs, small and large intestines but not in the oesophagus (149). The fact that the LRS may trap the scrapie agent has been suggested, allowing continuous amplification, hence higher exposure to the nervous targets, such as visceral autonomic fibres (1, 84).

Until very recently dissemination of the scrapie agent throughout the body via blood could not be substantiated in the natural disease. A number of attempts to identify the agent in the blood and serum of sheep and goats have been unsuccessful (51, 53, 54, 109). Preliminary results involving the transmission of natural scrapie via whole blood transfusions and a buffy coat transfusion clearly demonstrate the presence of infectivity in the blood (74). In this study, 21 sheep were transfused with blood taken from naturally scrapie-infected animals. Three of those which received whole blood from incubating donors developed clinical disease. A fourth sheep also developed clinical disease after receiving a buffy coat transfusion from a clinical donor. Other results are pending (74).

It is important to note that failure to detect the agent in certain tissues, secretions or excretions of sheep and goats, does not necessarily indicate its absence. It may exist in such low amounts that the chosen methods of detection are not sensitive enough. This is readily demonstrated by recent transmission studies.

Andreoletti and colleagues performed a detailed study with naturally infected Romanov sheep to better identify which cells and tissues support early replication of the scrapie agent and allow dissemination to other peripheral tissues and the CNS (1). This study suggests that early scrapie infection occurs via the oral route with the ileal Peyer’s patch as a likely site of entry. Heggebo and colleagues also found that PrP Sc was first detected in the Peyer’s patches and gut-associated lymphoid tissue (60). Replication and dissemination of the agent throughout the secondary LRS then take place via a lymphatic/vascular pathway. Infection of the gut-associated lymphoid tissues provides the opportunity for the infection of the autonomic nervous system which in turn allows the agent to migrate to the CNS (1). There is anatomical support for the ileal Peyer’s patch being the primary site of entry. Epidemiological observations suggest that very young ruminants are more susceptible to TSE infections. These observations are consistent with the known differences between young and old ruminants in the activity of their gut-associated lymphoid tissue and the passage of macromolecules across the gut wall (81).

This does not eliminate the possibility of scrapie infection by other routes of entry. Those which have been shown to be effective experimentally are scarification (130) and via the conjunctiva (57). A paper by Taylor et al. showed skin scarification to be as efficient and effective a route of scrapie infection in immunocompetent mice as intraperitoneal, intravenous or percutaneous inoculation (135).

**Distribution of infectivity**

With limited exceptions, the various studies performed by Hadlow and colleagues provide the primary bases for the determination of levels of infectivity in the different tissues of naturally scrapie-infected sheep and experimentally infected goats (51, 52, 54). This is important as there are now additional tissues where infectivity has been shown to exist, yet the titres have not been established.

Hadlow and colleagues examined the first appearance and temporal distribution of the scrapie agent in clinically normal scrapie exposed Suffolks of various ages, clinically affected adult Suffolks, a few clinically affected sheep of other breeds and clinically normal sheep over 54 months of age (52, 54). They also examined the distribution and concentration of infectivity in goats inoculated with scrapie by the intracerebral (i.c.) and...
subcutaneous (s.c.) routes (51). Infectivity titres were measured in mice which were inoculated intracerebrally. Another study using naturally infected Suffolk sheep, examined levels of infectivity in peripheral nerves (50). Tables I and II show the relative levels of infectivity in these studies. Table III displays additional tissues which have been shown to be infective but where no titres were obtained. Table IV shows negative research results which examined the possibility of infectivity in secretions or excretions.

**Table I**
Relative infectivity of tissues from Suffolk sheep naturally infected with scrapie

<table>
<thead>
<tr>
<th>Relative infectivity</th>
<th>Tissues</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest levels</td>
<td>Brain, spinal cord</td>
<td>54</td>
</tr>
<tr>
<td>Moderate levels</td>
<td>Lymph nodes (retropharyngeal, mesenteric-portal, pre-scapular, pre-femoral, etc.), spleen, tonsil, ileum, proximal colon</td>
<td>54</td>
</tr>
<tr>
<td>Low levels</td>
<td>Cerebral spinal fluid (CSF), sciatic nerve, pituitary gland, nasal mucosa, adrenal gland, distal colon, pancreas, liver, bone marrow, thymus, supramammary lymph node</td>
<td>54</td>
</tr>
<tr>
<td>No detectable infectivity</td>
<td>Blood clot, mandibular and parotid salivary glands, thyroid, heart, lung, kidney, skeletal muscle, mammary gland, testis</td>
<td>54</td>
</tr>
</tbody>
</table>

It is important to note that the above levels of infectivity reflect titres at the clinical stage of disease. In the preclinical stage of the disease the titres in the lymphoreticular tissue are actually higher than those in the central nervous system.

**Table II**
Relative infectivity from goats experimentally infected with scrapie (intracerebral and subcutaneous inoculation)

<table>
<thead>
<tr>
<th>Relative infectivity</th>
<th>Tissues</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest levels</td>
<td>Brain*, spinal cord</td>
<td>51</td>
</tr>
<tr>
<td>Moderate levels</td>
<td>Retropharyngeal, pre-scapular, pre-femoral lymph nodes, spleen, tonsil, adrenal gland*</td>
<td>51</td>
</tr>
<tr>
<td>Low levels</td>
<td>Cerebral spinal fluid (CSF)<em>, sciatic nerve</em>, pituitary gland*, nasal mucosa, ileum, proximal colon, distal colon, liver, thymus, mediastinal-bronchial lymph nodes, mesenteric-portal lymph nodes, parotid salivary gland*</td>
<td>51</td>
</tr>
<tr>
<td>No detectable infectivity</td>
<td>Blood clot, submaxillary salivary gland, thyroid, heart, lung, kidney, skeletal muscle, bone marrow, pancreas, ovary, saliva</td>
<td>51</td>
</tr>
</tbody>
</table>

* Pattison and Millson also detected infectivity (109)

Interestingly, as noted previously, a 55-month clinically affected Montadale had no detectable infectivity in any peripheral tissues examined (52). It should be noted that all of the studies by Hadlow were performed prior to knowledge of the genetic influence of the prion gene.

In addition to research detecting infectivity, a number of studies have now demonstrated the presence of PrPSc in the placenta (2, 114, 140, 141). More recent work is beginning to provide a better understanding of the progression of PrPSc accumulation.

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**Table III**
Additional tissues shown to harbour scrapie infectivity with no titres determined

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Species</th>
<th>Route of transmission and bioassay species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placenta</td>
<td>Sheep</td>
<td>Intracerebral inoculation to sheep and goats</td>
<td>110, 111</td>
</tr>
<tr>
<td>Placenta</td>
<td>Sheep</td>
<td>Intracerebral inoculation to mice</td>
<td>98</td>
</tr>
<tr>
<td>Placenta</td>
<td>Sheep</td>
<td>Intracerebral inoculation to mice</td>
<td>114</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>Goat</td>
<td>Intracerebral inoculation to goats</td>
<td>109</td>
</tr>
<tr>
<td>Blood</td>
<td>Sheep</td>
<td>Intravenous inoculation to sheep</td>
<td>74</td>
</tr>
<tr>
<td>Muscle</td>
<td>Goat</td>
<td>Intracerebral inoculation to goats (one transmission out of fourteen recipients)</td>
<td>109</td>
</tr>
</tbody>
</table>

(a) Hadlow et al detected infectivity in the parotid salivary gland but not in the submaxillary salivary gland (51)
(b) Pattison and Millson and Hadlow et al. did not show infectivity in blood; however the conditions of the bioassays were different (51, 53, 54, 109)
(c) Hadlow et al. did not detect infectivity in skeletal muscle tissue using different bioassay conditions (51, 54)

**Table IV**
Secretions/excretions where no scrapie infectivity was detected

<table>
<thead>
<tr>
<th>Secretion/excretion</th>
<th>Species</th>
<th>Route of transmission and bioassay species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces</td>
<td>Sheep</td>
<td>Intracerebral inoculation to mice</td>
<td>54, 64, 65</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>Intracerebral inoculation to mice</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intracerebral inoculation to a goat</td>
<td>108</td>
</tr>
<tr>
<td>Urine</td>
<td>Sheep</td>
<td>Intracerebral inoculation to mice</td>
<td>64, 65</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>Intracerebral inoculation to mice</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intracerebral inoculation to goats</td>
<td>108, 109</td>
</tr>
<tr>
<td>Saliva</td>
<td>Sheep</td>
<td>Intracerebral inoculation to mice</td>
<td>54, 64, 65</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>Intracerebral inoculation to mice</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intracerebral inoculation to a goat</td>
<td>108</td>
</tr>
<tr>
<td>Colostrum</td>
<td>Sheep</td>
<td>Intracerebral inoculation to mice</td>
<td>54, 64, 65</td>
</tr>
<tr>
<td>Milk</td>
<td>Sheep</td>
<td>Intracerebral inoculation to mice</td>
<td>64, 65</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>Intracerebral inoculation to mice</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intracerebral inoculation to goats</td>
<td>108</td>
</tr>
<tr>
<td>Semen</td>
<td>Sheep</td>
<td>Intracerebral to mice</td>
<td>64, 65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subcutaneously to sheep</td>
<td>104</td>
</tr>
</tbody>
</table>
in the placentome throughout pregnancy. The trophoblastic cells appear to be the primary site of PrPSc deposition although further work is required to provide a better understanding of the cellular and molecular basis of this mechanism. These studies are also beginning to offer valuable information which should be helpful in preventing the transmission of scrapie, as follows:

a) PrPSc was not present in uterine tissues from non-pregnant scrapie infected ewes (141)

b) PrPSc accumulation in the placenta appears to be controlled by polymorphisms of the foetal PrP gene (there was an apparent absence of PrPSc in placentas carrying foetuses encoding at least one ARR allele) (2, 141)

c) no apparent PrPSc was found within the foetus (2) (this concurs with findings in earlier studies [54, 65] and supports scrapie transmission as a parturient or post-parturient event).

Use of certain tissues for preclinical diagnosis

Studies conducted in the Netherlands and USA indicate that the immunohistochemical technique to detect PrPSc appears to be useful in detecting scrapie in preclinical sheep. This research has revealed the presence of PrPSc in the tonsils of preclinical sheep (119) and lymphoid tissue of the third eyelid (101, 102). The studies conducted in the Netherlands found that PrPSc staining in the tonsils was more consistent than other lymphoreticular tissues tested. In fact, in the most ‘susceptible’ genotypes, PrPSc was found in the tonsil at four to five months of age (121). The one drawback to using the tonsil as a site for a live animal test is the difficulty of collection. A general anaesthesia is used in all but very young animals. This not only adds additional costs to the procedure but brings the inherent risks of using general anaesthetics on ruminants. If, after careful evaluation, the tonsil remains the most reliable tissue for evaluation, post-mortem slaughter collection is a possibility for surveillance. Post-mortem collections of retropharyngeal lymph nodes or other possible lymph nodes are also possibilities for active surveillance programmes.

Testing the lymphoid tissue of the third eyelid for PrPSc can be performed by using a topical anaesthetic or post mortem. For testing the live animal, adequate restraint is a necessity. Recovery is usually uneventful. Detection of PrPSc in the third eyelid by IHC is usually found at 14 months of age (102). In the USA, the use of third eyelid biopsies has been incorporated into the scrapie eradication programme as an active flock monitoring tool. The methodology may yield suitable samples for approximately 80% of the sheep tested (103). On a practical basis, this is probably an overestimation as the sampling rate is greatly dependent upon the experience of the collector (D.L. Sutton, personal communication). The technique has been shown to be useful in identifying infected flocks which may have been exposed to scrapie but in which clinical disease has not yet been confirmed. There may be false negatives with the test if inadequate follicles are collected. This may be an inherent problem due to the age or breed of sheep or a technical collection complication. As mentioned previously, certain sheep, perhaps due to a genetic influence, may become infected with scrapie and progress to clinical disease without having detectable PrPSc. In these cases, the third eyelid would also not have detectable PrPSc (103).

The ability to detect PrPSc from placenta raises the possibility of using this tissue in a preclinical ante-mortem scrapie test.

The confirmation of scrapie infectivity in the blood provides a greater chance for success in finding an ante-mortem diagnostic test using blood. Capillary electrophoresis (CE) has shown some promise. The method involves a competition assay for PrPSc using fluorescent tagged peptides from the PrP and a specific antibody to the peptides (116). Other studies are also in progress to explore additional methodologies for the detection of PrPSc in peripheral blood or the possibility of using surrogate markers for the disease.

The reported detection of PrPSc in urine of TSE-infected animals may also provide a feasible diagnostic test (122). A number of laboratories are now in the process of repeating this work.

It probably cannot be emphasised enough that failure to detect the agent in certain tissues, secretions or excretions of sheep and goats in any one study does not necessarily indicate absence, as the agent may exist in such low amounts that detection methods are insufficiently sensitive. The recent blood transmission studies have demonstrated the importance of route, amount and bioassay species. Previous studies researching blood and its components involved i.c. inoculations to mice. There is a limit as to the amount of blood which can be injected into a mouse. In addition, a number of studies were performed without the knowledge of genetic influence and peripheral distribution. These examples all illustrate the need for further research.

Genetic influence

Genetic influence over the incidence of scrapie was demonstrated following the establishment of two lines of Cheviot sheep at the Neuropathogenesis Unit (NPU) in Scotland, which differed in incubation period after experimental infection with a source of scrapie called SSBP/I (28). The incubation time appeared to be controlled by a single gene, termed Sip (for Scrapie incubation period), with two alleles, sA (short) and pA (prolonged) (31).

Subsequently, studies of scrapie infectivity in hamsters led to the discovery of the PrP and the suggestion that it is the aetiological agent of scrapie (112), and then to the identification of the gene that encodes the PrP (96). This gene was shown to be congruent to Sip in sheep (93).
In recent years, Sip genetics has been entirely superseded by PrP genetics. An exhaustive study of the NPU Cheviot sheep and other flocks has revealed three polymorphic codons in the PrP gene with a strong link to the incidence and incubation period of scrapie (67). At codon 136, valine (V) was linked to scrapie susceptibility while alanine (A) was linked to resistance (68, 69). At codon 171, glutamine (Q) and histidine (H) were linked to susceptibility while arginine (R) was linked to resistance (67, 75, 99, 153). At codon 154, histidine (H) was linked to susceptibility while arginine (R) was linked to resistance (82).

This complexity is slightly misleading as, of the twelve possible combinations of these polymorphisms, only five appear to occur with any frequency (8, 73, 80). These five are alanine at codon 136, arginine at codon 154 and arginine at codon 171 (A<sub>136</sub>R<sub>154</sub>R<sub>171</sub> or ARR) and, continuing this notation, ARQ, AHQ, ARH and VRQ. These five alleles combine to give a total of fifteen possible PrP genotypes found in sheep.

**Genotype specific attack rates**

The relationship between the alleles/genotypes on the one hand and susceptibility to scrapie on the other is complex. Crude estimates of the relative susceptibilities of all fifteen genotypes are given in Table V and are expressed as scrapie cases per year.

**Table V**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases per million</th>
<th>National Scrapie Plan class</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARR/ARR</td>
<td>0</td>
<td>A</td>
</tr>
<tr>
<td>ARR/AHQ</td>
<td>0.3</td>
<td>B</td>
</tr>
<tr>
<td>ARR/ARQ</td>
<td>0.4</td>
<td>B</td>
</tr>
<tr>
<td>ARR/ARH</td>
<td>0</td>
<td>B</td>
</tr>
<tr>
<td>ARH/AHQ</td>
<td>5</td>
<td>C</td>
</tr>
<tr>
<td>ARH/ARQ</td>
<td>9</td>
<td>C</td>
</tr>
<tr>
<td>ARQ/ARH</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>ARQ/ARQ</td>
<td>5</td>
<td>C</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td>37</td>
<td>C</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td>6</td>
<td>D</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td>0.7</td>
<td>E</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td>225</td>
<td>E</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td>405</td>
<td>E</td>
</tr>
<tr>
<td>VRQ/VRQ</td>
<td>545</td>
<td>E</td>
</tr>
</tbody>
</table>

A : Sheep that are genetically most resistant to scrapie
B : Sheep that are genetically resistant to scrapie, but will need careful selection when used for further breeding
C : Sheep that have little genetic resistance to scrapie but may be sold or used for breeding for a specified future period
D : Sheep that are genetically susceptible to scrapie but may exceptionally be used for further (controlled) breeding in the context of an approved breeding programme
E : Sheep that are highly susceptible to scrapie and should not be used for breeding. Rams must be humanely slaughtered or castrated

and per million sheep of each genotype in Great Britain. It is important to remember that there may be fewer or many more than one million sheep of the genotypes in Great Britain and the numbers of scrapie cases shown in Table V are not those actually reported. The values were obtained by simply combining the proportion of all scrapie cases by genotype, with estimates of the proportions of these genotypes in the national flock. No consideration was paid to under-reporting (61), which is assumed to be unbiased with respect to genotype, and the process ignores some very important complexity, in particular differences among sheep breeds in genotype susceptibilities and frequency. Despite these limitations, the results are very enlightening.

The high degree of susceptibility associated with the VRQ allele is immediately apparent. The greatest attack rates by far are for the ARQ/VRQ, ARH/VRQ and VRQ/VRQ genotypes, with 225 to 545 reported cases per million per year. The resistance conferred by the ARR allele is also clear. The ARR/ARR genotype is the only numerically significant genotype (approximately 20% of sheep in Great Britain) for which no scrapie cases have been reported and the attack rate for the ARR/VRQ genotype (6 reported cases per million per year) is only a fraction of that of the three VRQ-encoding genotypes listed above.

After these three VRQ-encoding genotypes, the next most susceptible genotype (averaging across all sheep breeds in Great Britain) appears to be ARQ/ARQ, with 37 reported cases per million per year. Numerically, this genotype is one of the most common in Great Britain and, overall, the actual numbers of reported scrapie cases that are ARQ/ARQ are very similar to the numbers that are VRQ/VRQ. The susceptibility associated with the ARQ allele is also apparent from the high attack rate of the ARQ/VRQ genotype and, given the relative frequency of ARQ/VRQ in Great Britain, more than 50% of reported scrapie cases are ARQ/VRQ. Although the frequency of this allele in Great Britain appears to be ARQ/ARQ, with 37 reported cases per million per year, this number is VRQ/VRQ genotype and, given the relative frequency of ARQ/VRQ in Great Britain, there have been very few reported scrapie cases, resulting in an estimated attack rate of less than 1 reported case per million per year.

The effects of the AHQ and ARH alleles are subtle. The AHQ allele appears to confer resistance, and this is most apparent from the remarkably low attack rate estimated for the AHQ/VRQ genotype. Although the frequency of this allele in Great Britain is low and, accordingly, there may be considerable effects of sampling error in the calculations, it may be significant that the ARH allele only the attack rate for the homozygote (AHQ/AHQ) is greater than that of the VRQ heterozygote (AHQ/VRQ).

In combination with VRQ, the ARH allele clearly confers susceptibility and, indeed, the ARH/VRQ attack rate is the second greatest. However, in other genotypes it appears to confer resistance. Thus, the attack rate of ARQ/ARH is less than...
that of ARQ/ARQ, and that of AHQ/ARH is less than that of AHQ/AHQ.

**Genotype and scrapie incubation period**

The mean incubation period of scrapie tends to vary with PrP genotype (82). Generally, there is a negative relationship between the susceptibility of a genotype and the incubation period of the disease or, for animals where the date of infection is not known, their age at death. Thus, VRQ/VRQ scrapie cases tend to die younger than VRQ/ARQ cases which, in turn, die younger than ARR/VRQ cases.

**Breed effects**

As stressed earlier, the attack rates shown in Table V are only approximations, as much complexity has been overlooked in their calculation. In particular, there are very strong breed effects, such that genotypes that are highly susceptible in some breeds may be less susceptible, or resistant, in others. The most frequently cited example concerns the Suffolk breed. Suffolk sheep appear to lack the VRQ allele and the ARQ/ARQ genotype is the most susceptible to scrapie (71, 72, 96). Breed effects are not restricted to comparisons of breeds that do or do not encode VRQ, however. Both the ARQ/ARQ and ARR/VRQ genotypes appear to be resistant to scrapie in the NPU flock of Cheviot sheep (69), in French Romanov sheep, the VRQ/ARR genotype is resistant but the ARQ/ARQ genotype is susceptible (35) and, in Texel sheep in the UK, both ARR/VRQ and ARQ/ARQ genotypes are susceptible (6).

**Effects of scrapie strain**

Several different strains of natural scrapie have been identified on the basis of their characteristics in mice (15, 16), and some strains appear to attack genotypes differently. The NPU Cheviot sheep of the ARQ/ARQ genotype are considered to be resistant to scrapie following challenge with the scrapie strain SSBP/1 (46) and are resistant to natural scrapie that circulates in the flock (69). However, these animals have been shown to be susceptible to an isolate of scrapie, called CH1641, that was isolated from a natural scrapie case in the flock in 1970 (44). Therefore, in this instance, sheep of the same genotype and breed, and from the same flock, are susceptible to some strains of scrapie but resistant to others.

**Bovine spongiform encephalopathy in sheep**

The PrP genetics of BSE in sheep differs from that of most strains of natural scrapie (6). All known BSE infections of sheep have been obtained under experimental conditions and, accordingly, the total number of affected animals is fairly low. However, from the limited experimental data, it has become clear that the ARQ/ARQ, ARQ/AHQ and AHQ/AHQ genotypes are highly susceptible to BSE and the incubation periods are short (44, 77).

The effect of VRQ on susceptibility to BSE is currently unclear, as few VRQ-encoding sheep appear to have been challenged. The incubation period is short in ARQ/VRQ sheep inoculated directly into the brain, indicating a possible high susceptibility of this genotype, but the incubation period of A*Q/A*Q counterparts (where * can be R or H) may be shorter still (4, 7).

As with scrapie, the ARR allele is reported to confer some resistance to BSE and incubation periods are certainly long in ARR-encoding sheep (4). The status of the ARR/ARR genotype in regard to BSE is very unclear. For many years, this genotype has been considered to be entirely resistant to BSE and no animals have yet succumbed following oral exposure (77). However, it has recently been shown that sheep of this genotype are, in fact, susceptible to BSE following inoculation directly into the brain (66). It remains to be seen whether orally exposed ARR/ARR sheep are truly resistant to BSE or whether the incubation period is simply longer than the duration – so far – of the investigations underway.

**Genetic influence in goats**

In goats, polymorphisms of the gene encoding the PrP have been found at codons 21, 23, 49, 142, 143, 154, 168, 220 and 240 (9, 47). To date, only a couple of these appear to be linked to scrapie. A dimorphism at codon 142 (I → M) had previously been associated with an altered scrapie incubation time in goats (47). At codon 143 (H → R), the occurrence of scrapie may be associated with HH, as 13 of 15 cases of natural scrapie in a goat flock in Greece were HH, as opposed to HR or RR. The other two cases were HR. The HR cases did not display evidence of clinical disease and did not have evidence of histological changes. These cases had detectable PrP* in the brain. In the same flock, H at 154 may protect against scrapie, relative to R at codon 154 which would be similar to sheep (9).

Another PrP variant containing three instead of the usual five octapeptide repeats may also be associated with scrapie incubation time (48). As surveillance efforts detect more cases of scrapie in goats, further information regarding genetic influences should be obtained. Additional research is also required in this area.

**Transmission**

It has been effectively established that scrapie is an infectious disease which does not spontaneously arise from certain PrP genotypes (70, 73). However, as described above, genetic variations among sheep appear to determine whether the sheep will become infected and how quickly clinical signs may appear. Genetics may govern full susceptibility and resistance. Transmission occurs within flocks and between flocks. This section will summarise the following:

- past research regarding transmission
- new scientific findings on the mechanics of transmission
- epidemiological observations
measures which may prevent the maintenance of infection within a population of sheep or goats and areas where further knowledge is needed to improve the prevention, control and eradication of scrapie.

Discussions of scrapie transmission often classify routes as horizontal (lateral), vertical or maternal. In this text, the terms will be used as defined below:

a) horizontal (lateral) transmission: the spread of infection from one animal to another through direct or indirect contact

b) vertical transmission: the spread of infection from parent to offspring from the time of fertilisation through embryonic and foetal development in utero or from the transfer of infected germplasm

c) maternal transmission: the spread of infection from a dam to its offspring either vertically or laterally during parturition or by close post-parturient association (since maternal transmission encompasses both horizontal and vertical routes, it will not be discussed as a separate issue).

Susceptible hosts

The section on genetics summarises past and current information regarding the role of genetics in scrapie infection. It is known that polymorphisms in the prion protein gene of sheep but not necessarily goats are linked to the occurrence of scrapie. Hence, the prevalence of certain genotypes of sheep may influence the risk of transmitting scrapie between flocks and within flocks (5, 132, 158).

It should also be noted that not all genetically ‘susceptible’ sheep develop scrapie when orally challenged or when residing in naturally infected flocks (44, 72, 100). This implies that there may be other factors which influence susceptibility such as additional genetic factors (69), variable conditions for oral exposure, such as rumen contents (100), maturity (65) and agent strain (45).

As mentioned above, there are sheep which become infected and progress to clinical disease with no apparent peripheral infectivity or PrPSc. Although it is clear that these animals are susceptible to scrapie, it is not clear if they are a source of agent for other sheep.

Horizontal transmission

Historically, scrapie has been shown to be transmitted horizontally under field conditions between unrelated sheep (11, 30, 65). Evidence of lateral transmission is also displayed by goats that contract the disease after being reared with scrapie-infected sheep flocks (9, 11, 65, 139). Current studies showed that direct horizontal transmission is likely to account for the majority of scrapie cases in heavily infected flocks (92, 158).

As reviewed in the pathogenesis section, the most likely route of scrapie infection occurs via the oral route with gut-associated lymphoid tissue as the most likely site of entry. An oral route of infection is substantiated by experimental transmission of scrapie to sheep and goats by that route (43, 108, 110, 111). Other potential routes of natural infection which have been shown to be effective experimentally are scarification (130, 135) and via the conjunctiva (57).

Replication and dissemination of the agent throughout the secondary LRS then takes place through a lymphatic/vascular pathway. Over time the agent spreads to most lymph nodes with evidence that it invades a number of other non-neural tissues. Replication/propagation usually continues peripherally for months before evidence of infectivity can be detected in the brain and prior to the onset of clinical disease (1, 2, 34, 51, 52, 53, 54, 149).

Excretion/secretion of agent

In order for scrapie to spread to other sheep or goats the agent must be excreted and/or secreted. The long incubation period between exposure and clinical disease may allow the animal to shed the agent for an extended period of time. The precise source(s) of the agent and time when excretion/secretion begins have not been defined. The detection of infectivity in placenta (64, 98, 110, 111, 114), in combination with a failure to detect infectivity in faeces, saliva, urine, colostrum or milk (53, 54, 65, 108), has led to a fairly wide acceptance that the placenta plays a role in the spread of scrapie. In addition, a number of studies have now detected the presence of PrPSc in the placenta (2, 114, 140, 141). Hence, transmission would most likely occur from an infected mother to her progeny and other lambs that are in close association around the time of parturition. Whether this spread would be from direct contact with the infected tissues or from a contaminated environment or both is unknown.

It is important to note two aspects of placental infectivity, as follows:

a) occurrence in the incubation stage of the disease, thus allowing for exposure of susceptible animals and the environment over a longer period of time

b) this is not a constant event with every pregnancy.

Race and colleagues showed that PrPSc and infectivity were present in placentas from infected ewes during the preclinical phase of the disease (114). They also found that although a placenta may be positive during one pregnancy it may be negative during the subsequent gestation. Very recent studies have begun to provide a possible explanation for this observation. They demonstrated that PrPSc accumulation in the placenta is controlled by polymorphisms of the foetal PrP gene. In both projects, there was an apparent absence of PrPSc in placentas carrying foetuses encoding at least one ARR allele (2, 141). This aspect needs to be explored as it may provide an avenue to reduce the amount of agent supplied by the placenta.
As current understanding of the pathogenesis and genetics of scrapie continues to expand and the methods of detection improve, the potential for other routes of horizontal transmission needs to be revisited and studied in further detail. Although infectivity has not been detected in faeces, saliva, urine, colostrum or milk, most of this research was conducted before knowledge of genetic influence and with limited numbers of bioassay models (53, 54, 65, 108). Other findings as detailed in the pathogenesis section reiterate the need to conduct additional research to determine infectivity in these substances, as follows:

- the intestinal tract is an early site of replication (why would it not serve as a source for agent to be excreted in the faeces?)
- infectivity has been found in the salivary glands of scrapie infected goats (why would saliva not contain infectivity?)
- as infectivity has recently been detected in blood, the cellular components of colostrum/milk may suggest that this could be a source of infectivity under certain circumstances such as mastitis
- infectivity has been detected in small amounts in nasal mucosa (could nasal discharge possibly serve as a source of infectivity?)
- PrP has reportedly been detected in urine (122) (however, in this study it was not found to be infectious), these findings warrant additional attention.

Environmental contamination

Research which continues to show that infectivity is associated with the placenta strengthens the argument for environmental contamination through this source. Additional sources may exist. The extent to which scrapie is transmitted from a contaminated environment, including pastures, pens, barns, feed, water, bedding and other fomites (inanimate objects) is unknown. The agent has been demonstrated to survive in the environment for a number of years (14) and has remarkable resistance to inactivation (134). Consequently, indirect transmission is a possibility.

As studies to investigate the role of the environment are extremely costly and time-consuming, they have not been conducted, although some are planned in the UK. There is anecdotal evidence from Iceland that sheep that were restocked on premises that had been disinfected and left vacant for several years, developed scrapie (105, 106, 123).

Other sources of infection have been explored to explain the re-infection of sheep in Iceland. These possible sources include transmission by vectors or fomites. Hay mites may have played a role in the reoccurrence of scrapie (156) and gastrointestinal parasites may serve as a predisposing factor for scrapie infection (35, 83) or possibly a source of infection (65). There has been one unsuccessful attempt to transmit the disease by i.c. inoculation of Haemonchus contortus (39).

Vertical transmission

Germplasm

Several research projects have studied the transmission of scrapie in sheep by embryo transfer. The experiments have revealed conflicting results. Two studies performed in Scotland showed that offspring derived from both washed and unwashed embryos developed scrapie (41, 42). In the earlier study, six of twenty-six lambs were diagnosed with scrapie (41). These lambs were derived from unwashed embryos. The study completed in 1996 suggests that there is no obvious difference between the incidence of scrapie in groups of lambs from washed embryos (five of seven) and unwashed (five of six) embryos (42). The results of the 1996 study are difficult to interpret as offspring from uninoculated ewes also developed scrapie. This research project was conducted in such a manner as to take great precautions to prevent external contamination, yet the authors’ own admission is that this is still a possibility. Other possibilities for transmission in this study are the following:

- a carrier state in which the ewe does not exhibit signs of scrapie but is still capable of spreading the agent, or
- scrapie was transmitted through the seminal fluids or the spermatozoa.

Both rams used for the trial developed scrapie eight months after collection.

Another study involving embryo transfer was performed in the USA by Foote and colleagues (40). This study which only involved washed embryos did not result in the transmission of scrapie to progeny through the embryo or uterus. Due to the protocol followed in this study it was not possible to determine the dams of the resulting embryo transfer progeny. Given that only 30% to 61% of the inoculated donors developed scrapie, actual exposure to the agent is questionable. In addition, whether the length of time between inoculation and collection was adequate to allow exposure to the agent was questioned.

The most recent study reported in the USA involved the collection of embryos from exposed ewes originating from naturally infected flocks. All embryos were washed in accordance with standards set by the International Embryo Transfer Society. In this study, 17 donor ewes were diagnosed positive for scrapie. Five of the 17 were confirmed by both histology and IHC. The remaining 12 donors were only confirmed by one of the methods. These donors supplied embryos which resulted in 52 lambs. Of these, 33 offspring survived to 60 months of age. The 5 donors which were confirmed by both tests had 11 offspring which survived to 60 months of age. None of the offspring displayed any evidence of scrapie (150). The majority of donors and offspring in the study were AA 136QQ171. Scrapie is most often observed in this genotype in USA Suffolks (151). This study supplies additional
evidence to support the premise that there is little, if any, risk of transmitting scrapie through washed embryos. The authors did not supply individual donor information on age at collection, clinical history and the age that scrapie was diagnosed. This information should be evaluated to ensure that embryos were not collected at a very early stage of the incubation period, thereby affecting the potential distribution of infectivity.

The existing studies do not provide an adequate basis to confirm that embryo transfer is a truly safe method of preventing the transmission of scrapie. Further work must be conducted to provide a definitive answer as to embryo transfer and scrapie transmission.

To date, semen is thought to have little or no significance in the actual transmission of scrapie. The failure to detect the agent in semen, testes and seminal vesicles appears to indicate a lack of infectivity in semen (54, 64, 104). In the most recent embryo study described above (150), an immunohistochemically positive ram was used to breed two of the donor ewes. One of the donor ewes tested positive for scrapie, the other did not. There were eight offspring which resulted from the positive ewe and four from the negative donor. None of the offspring developed scrapie (150). However, this work involved a small number of progeny and genetic information was not specifically provided for the offspring of the donor that gave negative results for scrapie. Additional studies regarding semen and scrapie transmission are warranted.

**In utero transmission**

Work to date argues against the transmission of scrapie in utero. Limited studies have not detected infectivity in foetal tissues from naturally infected dams (54, 65) and no apparent PrP\textsuperscript{s}} was detected within the foetus (2). The anatomy of placental connections in sheep is intended to ensure isolation of the foetus from the chorionic structure. This may explain the absence of infectivity in the foetuses examined to date (40, 140). This may not be the case if the integrity of the foetal-maternal barrier is lost.

Epidemiological evidence suggests that if vertical transmission occurs at all, it does not account for the majority of cases in heavily infected flocks (35, 90, 158).

**Iatrogenic transmission**

There have been two instances where the use of a vaccine has been incriminated as the source of scrapie for a large number of cases. The first outbreak affected over 300 of several thousand sheep vaccinated with a louping ill vaccine. This vaccine was manufactured using formalinised sheep brains (49). The second was much more recent. In this case, a vaccine against Mycoplasma agalactiae is thought to be the source of scrapie that affected hundreds of sheep and goats in Italy (19, 20).

**Possibility of carriers**

Studies on the placenta support the theory that sheep in the preclinical phase of scrapie are a source of infection to other sheep and goats. It is unknown if certain animals may become carriers, i.e., become infected, shed agent but do not progress to develop clinical disease. Infection of certain rodent species with different TSE strains suggests the possibility of a carrier state (24, 113, 115). In the most recent study, mice were inoculated with 263K hamster scrapie. There was a prolonged period (approximately one year) where there was no evidence of replication of infectivity. Furthermore, there was no evidence of PrP\textsuperscript{s}} during this phase of inactive persistence. This study found that this phase was followed by a period of active replication of infectivity and agent adaptation. In most cases, PrP\textsuperscript{s}} was not detected in the active phase as well (115). It is important to determine if this persistence and adaptation occurs in natural sheep scrapie as it may have significance in breeding programmes to eradicate scrapie. For example, if a flock is heavily infected and the only method of control is breeding for ‘resistance’, it may be possible for the agent to persist and adapt in these new genotypes. Over time, the ‘resistant’ genotypes may become a source of agent.

Considering the results of the study by Race and colleagues, if there were an inactive persistence phase in sheep, PrP\textsuperscript{s}} would not be detectable, yet there would be infectivity (114). Given that the primary methods for scrapie diagnosis involve the detection of PrP\textsuperscript{s}}, if the persistence and adaptation were to occur in sheep, it may be overlooked for some time. Mathematical modelling of the transmission dynamics of scrapie show that a breeding programme would not be as effective if carrier states exist (158). Therefore, it is essential for research to continue in this area as a number of countries have begun to use genetic selection as a means of scrapie control.

Data from an analysis of natural scrapie in a closed flock indicated that ‘resistant’ animals did not appear to be carriers or at least were less infectious when comparing risk for lambs born to ‘resistant’ versus ‘susceptible’ dams (35). The study found that lambs born to dams which developed scrapie seemed to have a higher risk of developing the disease. All of the VRQ/VRQ progeny from dams dying of scrapie developed the disease, whereas only half of the VRQ/VRQ progeny from healthy dams became ill. This was not statistically significant which may be due to the small number of cases. However, when considering the genotype of the dam, the risk is lower for lambs born to resistant dams. Survival analysis revealed that when taking into account both dam status and dam genotype, the risk was higher for progeny from susceptible dams, even if the dams did not display evidence of clinical disease. This suggests that these animals were not carriers or were less a source of the agent (35).

**Public health**

There is no scientific evidence to indicate that scrapie poses a risk to human health (59, 79). There is no epidemiological

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evidence that scrapie of sheep and goats is transmitted to humans, such as through contact on the farm, at slaughter plants or from butcher shops (13). In a 1999 consultation, the World Health Organization reviewed the evidence and came to the same conclusion (159).

Clinical signs
The most important feature to remember about the clinical picture of scrapie is that a wide range of signs are involved. This cannot be stressed enough when educating producers. Many scrapie surveillance programmes around the world are passive, that is, they rely solely on reporting. Accurate and complete reporting requires owners to be knowledgeable of the full range of signs and be willing to report. Disease surveys have found that there is still considerable confusion regarding the clinical signs of scrapie despite educational efforts (61, 118). In the Netherlands, approximately 60% of respondents did not know or were not sure what scrapie was (118). In Great Britain, 18% of the farmers replying did not know what clinical signs were associated with scrapie and 15% would dip or inject any sheep they suspected of having scrapie (61). In a recently completed United States Department of Agriculture (USDA) survey of sheep producers, 7.4% of the respondents had never heard of scrapie and another 28.5% had only heard of the name (144).

Scrapie is a non-febrile, insidious disease of sheep and goats. Affected animals will usually show behavioural changes, tremor (especially of the head and neck), pruritus and locomotor incoordination which progresses to recumbency and death. The clinical course of scrapie is usually of a longer duration (one to six months) but on occasion may be one to two weeks (19, 44). Some studies have reported that sheep with scrapie may simply be found dead (23). The duration of clinical signs may also be dependent on the observational abilities of the attendant.

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 of the flock. In many instances, this passes unnoticed. Some sheep appear to be hyperexcitable and restless. Like sheep, goats may lose weight, display tremor and become ataxic. Affected goats are less likely to rub against fixed objects, but scratch vigorously with hind feet and horns (W.J. Hadlow, personal communication). In Italy where a large number of goats developed scrapie after what appears to be an accidental infection, additional clinical signs were noted in goats. As reported in cows with BSE (154), there was difficulty milking affected goats. Goats could also be aggressive and cannibalistic. Premature kidding and pica were also observed (19).

Scrapie occurrence
Within flocks
Scrapie occurs most frequently in sheep of either sex between two and five years of age (28, 123, 155). Cases of the disease are not common before 18 months (29, 123, 155). However, a few cases of natural scrapie have been reported in sheep at approximately one year of age (78, 161). Scrapie may occur in animals over 60 months of age (6, 19, 107, 155). There is a documented case of an animal eleven years of age (107). In many of the cases of older sheep developing scrapie, it is difficult to know if the incubation period is extremely long or if exposure occurred later in life.

Within-flock incidence can be extremely variable. Surveys of farmers in Great Britain and the Netherlands have found mean within-flock incidences of 0.37% and 1.2%, respectively (62, 118). Other observations have included within-flock incidences ranging between 1% and 20% (62, 123, 160).

Iatrogenic transmission has resulted in high mortality rates over relatively short periods of time within certain flocks (20).
Advancements in testing are beginning to assist the identification of preclinical cases of scrapie within flocks. In the USA, the use of third eyelid biopsies has been incorporated into the scrapie eradication programme as an active flock monitoring tool. This has proved to be a useful method for identifying infected flocks that may have been exposed to scrapie but in which clinical disease has not yet been detected and for identifying additional positive sheep within infected flocks (103).

Within countries

Until recently, limited diagnostic capabilities have precluded most countries from attempting to identify scrapie incidence within a country. The one exception was Iceland, where large-scale abattoir surveillance was conducted using histopathology of brain tissue. This surveillance began in 1978 and between 10,000 and 15,000 samples were tested each year. This surveillance was used to assist efforts to eradicate scrapie (123). Prior to the recent slaughterhouse surveillance using laboratory diagnosis, a number of countries used surveys in an attempt to ascertain an estimate of scrapie prevalence. Such surveys have been conducted in Great Britain, the Netherlands and the USA. The surveys have been conducted through the mail, by personnel interview on the farm or by questionnaires at events attended by sheep farmers (61, 62, 95, 118, 142, 143, 144).

In each country, estimates of incidence were determined. For the most part, each was dependent upon the ability of producers to make a fairly accurate diagnosis of scrapie based on their knowledge of clinical disease. There were two findings which were consistent among all of the surveys, as follows:

a) considerable confusion exists concerning the clinical signs of scrapie despite educational efforts (see ‘Clinical signs’)

b) scrapie was under-reported in these countries when comparing the estimated incidence obtained from the survey to actual cases reported.

The ability to test for scrapie using methods to detect PrPsc has stimulated a number of countries in addition to Iceland to conduct large-scale surveillance projects. A study was performed in Great Britain in 1997 and 1998 to determine occurrence and prevalence in sheep sent to slaughter. The sample population was 2,809 apparently healthy sheep, of which 55% were less than 15 months of age. Each sample was examined for histopathological changes associated with scrapie and scrapie-associated fibrils (SAF). Brain samples from 500 of the animals were also examined using IHC. Any sample which was positive or inconclusive by histology, SAF or IHC was tested by Western blot analysis. This study did not identify any unequivocally positive sheep (125). The fact that 55% of the samples came from sheep under 15 months of age probably contributed to this finding. A stochastic model was developed to estimate the prevalence of scrapie in Great Britain using the results of the abattoir surveillance (152).

Currently, the EU and the USA are conducting slaughterhouse surveillance to provide a more accurate estimate of the occurrence of scrapie and the geographical distribution. In the EU, scrapie testing was modelled after the active BSE surveillance. Between January and December 2002, 1,124 cases of scrapie were identified. The cases in either sheep or goats occurred in all member states of the EU with the exceptions of Austria, Denmark, Luxembourg, Portugal and Sweden (37).

A slaughterhouse surveillance project is underway in the USA. This is being conducted over a one-year period. The objective is to estimate national and regional prevalence. It will also serve as a pilot project to determine if this method of sampling would be a valuable tool in the national scrapie eradication programme.

Prevention and control

Risk factors

Identifying the risk factors associated with the spread of scrapie is essential if scrapie is to be eliminated not only within individual flocks but in countries as well. Epidemiological studies and mathematical models are now being utilised to supplement direct transmission and pathogenesis research. A combination of approaches will be required to provide answers to the many questions regarding scrapie and its spread. Studies have found that movement of animals, flock size, breeding practices and lambing management are some of the factors which may appear to influence the spread of scrapie.

The inadvertent introduction of infected preclinical sheep through the purchase of breeding animals is the most consistent risk factor for the introduction of scrapie (33, 62, 63, 65). In addition, sharing pastures or rams may also contribute to the introduction of scrapie (63).

Breeding and lambing management practices have also been identified as risk factors (35, 90). The anonymous postal survey conducted in Great Britain found that flocks which breed and raise many of their replacements are more likely to record the occurrence of scrapie. Furthermore, farms on which ewes lambed unconfined on pasture or in individual pens were 25% to 30% less likely to record a scrapie case than farms on which ewes lambed in group pens (90). This would be consistent with the findings of infectivity associated with placenta.

Other factors which influence the transmission and maintenance of scrapie within and between flocks are as follows:

a) The amount and duration of agent excreted and exposure to the agent: the amount and duration of agent excreted is dependent on incubation period, life span of the animal...
(55, 132) and possibly pregnancy status of the ewe (140). If the environment serves as a source of infection, exposure and maintenance may be prolonged depending on survivability of the agent (158).

b) The number of genetically susceptible sheep within a flock: this may result from breeding susceptible replacements or purchasing susceptible replacements (5, 55, 56).

Control programmes

There have been many different approaches to scrapie control and eradication. Countries such as Australia and New Zealand which have detected the disease after introduction but before widespread transmission have apparently been successful in eradicating the disease within a short period of time. Other countries in which the disease has become endemic, such as Canada, Iceland and the USA, have implemented various strategies in an effort to eliminate the disease. Programmes have been implemented in these countries for several decades with limited success. Iceland has made a significant effort and may soon be close to eliminating scrapie (136).

The emergence of BSE in 1986 and the experimental transmission of BSE to sheep and goats have prompted a number of other scrapie-endemic countries to initiate programmes to eliminate the disease. Approaches to scrapie control/eradication which have been utilised over the years are listed below:

- complete flock depopulation of infected and source flocks
- depopulation of certain genetically related animals (usually based on maternal transmission)
- 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
- the use of selective breeding programmes based on PrP genotype.

Genetic control of scrapie

The very strong association of scrapie with the PrP genotype has raised the possibility of controlling the disease, at a national level, by selective breeding (120). Indeed, genetic control programmes are now underway in several countries, including France, the Netherlands, UK and USA. Other countries such as Austria and Germany have genotyped the various breeds within the country in anticipation of using genetics as a tool to control scrapie (32, 126). Recent legislation in the EU provides a provision for keeping sheep of the ARR/ARR genotype in scrape-infected flocks to be culled (36). Some examples of national programmes are provided below. It is important to note that these programmes have been designed taking into consideration the situation in each country.

United Kingdom

The UK Government control programme (termed the ‘National Scrapie Plan’ or NSP) was launched in 2001 (26). The long-term aim is to eliminate all scrapie by increasing the frequency of the ARR allele in the national sheep flock to a level at which the disease cannot persist. In the short term, the aim is to reduce the occurrence of scrapie by promoting the use of ARR-encoding rams (with the exception of ARR/VRQ animals), and decreasing the frequency of the VRQ allele by castration or slaughter of all VRQ-encoding rams. The ARH, ARQ and AHQ alleles have a temporary stay of execution. The first phase of the programme is centred around the mass genotyping of rams and ram lambs in scrapie-free sheep flocks, on a voluntary basis and at no cost to the flock owner, with the aim of producing flocks that cannot become newly affected with scrapie. Furthermore, the scheme is currently restricted to pedigree and pure-bred flocks. It is hoped that the genotype-shift expected in these flocks will, in time, filter into other sectors of the sheep industry.

The genotype classification system of the NSP is shown in Table V. In broad terms, the NSP categorisation clearly corresponds to the estimates of scrapie cases per million per year, although ARR/VRQ and AHQ/VRQ are notable exceptions. The NSP classifications are defined in terms of risk to sheep of each genotype, but in reality the scheme takes a longer-term, allele-based approach. For example, the risk of scrapie in AHQ/VRQ sheep is very small, but progeny that inherit the VRQ allele may be at very high risk, depending on the second allele, and the AHQ/VRQ genotype must therefore be targeted for eradication.

Scrapie-affected flocks (from any sector) are being addressed separately in a second phase of the programme. The aims are to prevent further transmission of scrapie and reduce the risk of TSEs entering the food chain and, to achieve this, the ARR allele frequency must be increased rapidly. To this end, only ARR/ARR males may be used for breeding and, in most instances, breeding ewes must encode ARR and not VRQ. Clearly, this scheme requires PrP genotyping of all breeding sheep. This scheme, which will be voluntary under the NSP, is expected to become compulsory under EU law in 2003.

United States of America

The scrapie programme in the USA has a two-faceted approach. The Scrapie Flock Certification Program monitors flocks over time. The Certification Program is intended to assign status to flocks with no evidence of scrapie, thus providing a source of ‘clean’ breeding animals. This programme has requirements for identification, record-keeping, reporting and restrictions on flock additions. Certification is based solely on absence of disease, not on genetics. Certification is also intended for flock owners or breed associations who prefer to keep their flock free.
of the scrapie agent and not have to revolve their entire breeding programme around scrapie genetics.

The other facet to scrapie control in the USA is a programme to eliminate disease from infected and source flocks. There have been many modifications over the past fifty years. During the 1990s this aspect of scrapie control involved the elimination of high-risk animals from infected and source flocks. Risk was based on the possibility of scrapie exposure during lambing. Most recently the programme has been modified to incorporate a genetic component.

The following are taken from the National Institute for Animal Health educational material for producers:

a) Three basic steps in the new plan, as follows:
- when an infected flock has been identified, the sheep are genotyped; the genotype of the sheep determines its risk for scrapie
- high-risk genotypes are either removed or their movement restricted
- the flock is placed under surveillance for five years.

b) Benefits of the plan are listed below:
- in most cases, producers will be able to keep many more of their sheep with a genetics-based plan. This plan allows owners to retain or sell without restrictions nearly all sheep that are AA RR, AA QR and most AV QR (any AV QR that is likely to have been exposed to a strain to which it is susceptible is restricted) from infected or source flocks once owners have met certain conditions (Table VI).

All sheep that are AA RR, AA QR are restricted only in rare cases when the animal is:
- the female offspring of a female positive animal
- a clinical suspect
- from a flock with unusually high prevalence

Requirements of the Program
All exposed QQ ewes, exposed female goats (no comparable genetic research that shows which individual goats are resistant is available, therefore at this time all goats must be considered susceptible), and female offspring of scrapie positive ewes will be removed or will be subjected to movement restrictions.

Ewes of other genotypes may be required to be removed or may be restricted, depending upon epidemiological findings and the genotypes of other sheep testing positive.

In addition:

a) All animals in the flock must be identified officially and entered in the USDA Scrapie National Generic Database by federal and/or state personnel.

b) Owners must have a post-exposure management and monitoring plan that includes the following:
- official identification of sexually intact animals that are sold or acquired and records of such transactions, including information on the buyer/seller
- reporting of deaths of any mature animals and submitting animals that show possible signs of scrapie for diagnostic testing
- annual inspections by state and/or federal officials
- owners who elect to retain restricted female animals will have to meet additional requirements, including testing and restrictions on some offspring.

Other aspects of the Program
Owners whose animals must be removed from the flock will receive compensation from the federal government. Furthermore, the federal government will provide testing and pay part of the disposal costs.

The goal of the USA is complete elimination of scrapie.

A caveat to genetic selection
It is important to determine if agent persistence and adaptation occurs in natural sheep scrapie as it may have significance in breeding programmes to eradicate scrapie. For example, if a flock is heavily infected and the only method of control is breeding for ‘resistance’ it may be possible for the agent to persist and adapt in these new genotypes. Over time the ‘resistant’ genotypes may become a source of agent. Mathematical modelling of the transmission dynamics of scrapie show that a breeding programme would not be as effective if carrier states exist (158).

Table VI
The association between genotype and susceptibility/resistance to scrapie, as defined by the scrapie eradication plan of the United States Department of Agriculture

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Susceptibility/resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. AA RR</td>
<td>Sheep which are resistant</td>
</tr>
<tr>
<td>2. AA QR</td>
<td>Sheep which are rarely susceptible</td>
</tr>
<tr>
<td>3. AV QR</td>
<td>Sheep which are susceptible to some scrapie strains that are believed to occur with low frequency in the USA</td>
</tr>
<tr>
<td>4. AA QQ</td>
<td>Sheep which are highly susceptible</td>
</tr>
<tr>
<td>5. AV QQ</td>
<td>Sheep which are highly susceptible</td>
</tr>
<tr>
<td>6. VV QQ</td>
<td>Sheep which are highly susceptible</td>
</tr>
</tbody>
</table>
It is also important to determine if breeding solely for scrapie resistance will have a negative impact on other breed characteristics and production traits.

**Prevention**

The ideal means for preventing the introduction of scrapie is to maintain a closed flock, especially in regard to pregnant ewes. Any replacement ewes or breeding rams should originate from flocks not known to be affected with scrapie and have management practices precluding the introduction of scrapie. In reality, this is difficult to ensure since there is no definitive pre-purchase test to assure freedom from the disease. It would be possible to have the animal screened by a third eyelid or tonsil biopsy. However, the limitations of current tests do not preclude the possibility that an animal is infected and gives a negative result. The current preclinical tests are probably best used on a flock basis. A buyer must rely on the knowledge, integrity, and honesty of the seller.

Despite the lack of a definitive live animal diagnostic test, there are some preventive steps a potential purchaser or new owner of purchased animals may take, as follows:

1. Know the flock health status for the flocks in which an animal has been housed.

2. If a ewe of unknown or questionable disease status is present in the flock, spread to other animals may be minimised by keeping her separate from the rest of the flock at lambing time and for a period of time following lambing.

3. In regard to a test for scrapie susceptibility, current research shows that there is evidence to show certain genotypes may be resistant to scrapie infection or at least may reduce the transmission of scrapie. Flock status is still important as research has not eliminated the possibility of ‘carrier’ animals.

4. If the flock develops scrapie, the risk of further spread and/or reintroduction of the disease may be minimised through removal of high-risk animals, careful cleaning and disinfection of facilities, and improved management of animals at lambing time, paying particular attention to segregate animals into small groups and to keep the risk classification of animals in each group at the same level. In certain countries, the method of control is total depopulation of the infected flock and in some cases total depopulation of the source flock.

5. Although meat-and-bone meal contaminated with the scrapie agent has not been definitively proven to play a role in the transmission of scrapie, prohibition of the feeding of ruminant meat-and-bone meal to sheep and goats in countries with endemic scrapie or other TSEs is a prudent approach.

The above recommendations are suggestions which may assist in reducing the risk of introducing scrapie into a flock or region. Research must continue so that maintaining a closed flock is not the only guaranteed method of preventing scrapie. There must be a better understanding of routes of transmission, the period of infectiousness, the issue of a carrier state and environmental clean-up to prevent re-exposure. The development and validation of an early preclinical, logistically feasible live animal test would also allow significant progress.

**Treatment**

Currently there is no effective treatment for scrapie although research has been conducted in many different areas. These have included a wide variety of anti-infectious agents, immunomodulating drugs and chemicals. Some of these have prolonged incubation periods of laboratory animals but none have been effective in totally prohibiting the onset of disease and death (12, 21, 128, 133, 157).

There are also efforts to develop a vaccine as a means of both prevention and treatment. To date, the studies have shown a prolongation of incubation time but have not fully impeded the disease (124, 129).

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L’épidémiologie de la tremblante

L.A. Detwiler & M. Baylis

Résumé
Les auteurs dressent un bilan actualisé de l’épidémiologie de la tremblante. Les informations présentées abordent aussi bien les aspects historiques que les données les plus récentes concernant la détermination de la distribution géographique de la maladie, sa pathogénie, sa transmission, sa détermination génétique et sa dynamique au sein d’un troupeau. Les facteurs de risque associés à la tremblante et à sa transmission, au sein d’un troupeau et entre troupeaux, sont également décrits. Quelques programmes actuels de lutte contre la tremblante au niveau national sont évoqués à titre d’exemples.

Mots-clés

Epidemiología del prurigo lumbar

L.A. Detwiler & M. Baylis

Resumen
Los autores repasan la epidemiología del prurigo lumbar, ofreciendo datos históricos y recientes sobre la determinación de su distribución geográfica y la patogénesis, transmisión, influencia genética y dinámica de la enfermedad dentro de un rebaño. También exponen los factores de riesgo de prurigo lumbar y la propagación de la patología en el interior de los rebaños y entre ellos. Para acabar describen sucintamente una serie de programas nacionales de lucha contra la enfermedad que se están aplicando actualmente.

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