Scrapie

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Summary: A detailed review is presented of the history, geographical distribution, cause, epidemiology, clinical features, pathogenesis, pathology, diagnosis, prevention, control and economic effects of scrapie in sheep. Brief mention is made of the disease in goats and moufflon. The nature of the agent causing scrapie, the genetic control of the incubation period in sheep and the natural transmission of scrapie in sheep and goats are discussed. National efforts to control scrapie in various countries are outlined.

KEYWORDS: Disease control - Disease occurrence - Prion disease - Reviews - Scrapie - Sheep diseases - Spongiform encephalopathy.

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INTRODUCTION

Scrapie is an insidious, degenerative disease affecting the central nervous system (CNS) of sheep and goats. The disease is also called la tremblante (French: trembling), Traberkrankheit (German: trotting disease), or rida (Icelandic: ataxia or tremor); it is also known by numerous other names. The disease was first recognised as affecting sheep in Great Britain and other countries of Western Europe over 250 years ago.

Scrapie is the prototype of the group of diseases known as the sub-acute, transmissible spongiform encephalopathies which affect man and some animal species, notably ruminants. Of the three human spongiform encephalopathies in this group: kuru, Gerstmann-Sträussler-Scheinker (GSS) syndrome and Creutzfeldt-Jakob disease (CJD), GSS and familial CJD have a hereditary cause but are also transmissible. Host genes can exert a major effect on the length of the incubation period and on clinical disease occurrence in some of the diseases, including natural scrapie in sheep. The cause of the animal diseases and kuru, a geographically localised human disease, is a polymorphic transmissible agent which has yet to be characterised. However, all the diseases share the following characteristics:

a) a prolonged incubation period of months or years

b) a progressive, debilitating, neurological illness which is always fatal

c) pathological changes which are confined to the CNS and include vacuolation, neuronal loss, astrocytosis and, in some diseases or individuals, amyloid plaques may be seen

d) the presence of scrapie-associated fibrils (SAF) in detergent-treated extracts of brain tissue examined by negative stain electron microscopy

e) an absence of detectable inflammatory or immune responses.

While sheep, goats and moufflon (Ovis musimon) (133) are susceptible to natural scrapie, the disease occurs primarily in sheep of breeding age with an age of peak incidence at approximately three and a half years (100).

GEOGRAPHICAL AND TEMPORAL DISTRIBUTION

Scrapie has been reported world-wide and affects most sheep-producing regions with a few notable exceptions. The disease has been recognised for over two centuries in England, Wales and Germany (102).

Historical record of early outbreaks

The first reports of the existence of scrapie appear in eighteenth and nineteenth century literature from England and Germany. According to McGowan (85), the earliest definite record of the occurrence of scrapie in Britain was in 1732. Leopoldt (83) gave a graphic account of the disease in Germany. Between 1750 and the early 1800s, there are accounts of a scrapie-like disease occurring in the Dorset Horn, Wiltshire Horn and Norfolk Horn breeds in England. Reports of a scrapie-like
condition on the continent of Europe at this time primarily link scrapie to imported Spanish Merino sheep (102), although Greig (52) maintained that it most probably was endemic prior to the introduction of the Merinos.

Later, in England and Scotland there were reports of scrapie in the Border Leicester, Blue-Faced Leicester, Cheviot, Scottish Blackface and Oxford Down breeds. Between 1920 and 1950, scrapie became a major problem in the English Suffolk breed, causing considerable financial loss in some flocks. Concern about the disease in the 1930s led to the development of research at the Moredun Institute in Edinburgh (102). Since 1950, there have been numerous reports of field outbreaks. Although scrapie is not a notifiable disease in the United Kingdom, it is believed that the disease has affected most breeds. There is anecdotal evidence to suggest that the increase of scrapie in different breeds is a result of greater sheep movements, particularly in the post-World War II era.

Current occurrence of scrapie within countries

The importation of sheep from countries where the disease was endemic has led to the occurrence of scrapie in many countries, as shown in Table I. Studies by Hourrigan and colleagues (64) illustrate vividly the association between new occurrences of scrapie and prior movements of sheep.

### Table I

**Occurrence of scrapie following the importation of infected animals**

<table>
<thead>
<tr>
<th>Countries</th>
<th>Date</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iceland</td>
<td>1878</td>
<td>(116)</td>
</tr>
<tr>
<td>Canada</td>
<td>1938</td>
<td>(115)</td>
</tr>
<tr>
<td>United States of America</td>
<td>1947</td>
<td>(126)</td>
</tr>
<tr>
<td>New Zealand</td>
<td>1952</td>
<td>(6)</td>
</tr>
<tr>
<td>Australia</td>
<td>1952</td>
<td>(11)</td>
</tr>
<tr>
<td>Norway</td>
<td>1958</td>
<td>(102)</td>
</tr>
<tr>
<td>India</td>
<td>1961</td>
<td>(139)</td>
</tr>
<tr>
<td>Hungary</td>
<td>1964</td>
<td>(1)</td>
</tr>
<tr>
<td>Republic of South Africa</td>
<td>1966</td>
<td>(130)</td>
</tr>
<tr>
<td>Kenya</td>
<td>1970</td>
<td>(20)</td>
</tr>
<tr>
<td>Germany</td>
<td>1973</td>
<td>(61)</td>
</tr>
<tr>
<td>Italy</td>
<td>1976</td>
<td>(21)</td>
</tr>
<tr>
<td>Brazil</td>
<td>1978</td>
<td>(95)</td>
</tr>
<tr>
<td>Yemen</td>
<td>1979</td>
<td>(64)</td>
</tr>
<tr>
<td>Sweden</td>
<td>1988</td>
<td>(35)</td>
</tr>
<tr>
<td>Cyprus</td>
<td>1989</td>
<td>(127, 128)</td>
</tr>
<tr>
<td>Japan</td>
<td>1990</td>
<td>(96, 97)</td>
</tr>
</tbody>
</table>

*Outbreaks have also been noted in Austria, Belgium, Colombia, Czechoslovakia, Ireland, the Isle of Man, Lebanon, the Netherlands, Northern Ireland, Somalia, Switzerland, United Arab Emirates.*
The stringent and prompt efforts made in Australia and New Zealand to depopulate imported scrapie-infected sheep, as well as any contact animals, allowed these countries to successfully eradicate the disease. They are now considered by most countries to be scrapie-free.

It is difficult to develop all-inclusive guidelines which would establish scrapie-free status for a country. Currently, the criteria being used to establish country status are:

- mechanisms of reporting;
- diagnostic capability;
- past sourcing of sheep and goats;
- import restrictions; and
- previous reports of scrapie in the country.

The lack of a preclinical screening test for scrapie infection adds to the complexity of ascertaining freedom from the disease. For the same reason, it is virtually impossible to obtain a true picture of disease prevalence in a country where scrapie is endemic. An accurate measure of prevalence would require that all cases of scrapie be reported and submitted for a confirmatory diagnosis. Since many outside factors influence reporting, actual prevalence continues to remain obscure in most countries and it is rarely possible to justify freedom from scrapie on the basis of surveillance alone. There is strong pressure on individual farmers to conceal incidents of scrapie due to the disastrous trading consequences which may result from even one case being reported.

Several countries have made attempts to determine the national prevalence of scrapie. In Great Britain, where scrapie is not a notifiable disease, a self-administered questionnaire survey using two independent groups of respondents was conducted (94). One survey was conducted at a national sheep exhibition and the other was a postal questionnaire sent to members of the British Dairy Sheep Association. Producers were asked if they knew what scrapie was, if it was present in their flock and if so, how many cases there were and how was it diagnosed? They were also asked to identify the clinical signs of scrapie from a list of choices. The survey found the prevalence of scrapie to be 34.5% in the exhibition study and 17% in the Dairy Sheep Association. The authors point out various selection biases in the survey. One of the primary criticisms from outside sources was the use of producer speculation rather than diagnostic confirmation to estimate prevalence (87).

Iceland is making a monumental attempt to screen the national sheep population for evidence of scrapie infection as part of a long-term programme to eradicate the disease. Each year since 1978, between 10-15,000 brain samples (brain stems removed via the foramen magnum) have been submitted for histological examination. This is being carried out in the hope of identifying infected flocks. Samples were obtained from apparently healthy animals at slaughter and from clinically normal sheep in heavily infected flocks. Ten percent of the clinically normal sheep from the heavily infected flocks showed evidence of symmetrical neuronal vacuolation. In addition, fifteen infected flocks were identified through this surveillance effort prior to the appearance of clinical cases (116).

In some countries, the disease may be characterised by association with a certain breed, geographical location or type of farming operation. For example, in Iceland
the disease was confined to a limited section in the north for seventy years after it was introduced. During the mid-1900s the disease spread to other parts of the country (116). In Spain, scrapie is restricted to Aragon, a region in the north-east of the country which is near the French border (Badiola, personal communication).

Northern Ireland appears to have a much lower incidence of scrapie than the rest of the United Kingdom. The same holds true for the Republic of Ireland (Bradley, personal communication).

In the United States, approximately 84% (USDA/APHIS, Veterinary Services, unpublished statistics) of the cases diagnosed were Suffolk sheep. The cases of confirmed scrapie in the United States were animals raised in farm flocks, despite the fact that the majority of sheep are raised on the range. A farm flock can be defined as a group of sheep raised in a confinement or semi-confinement situation and lambed through a common facility. A range operation consists of large numbers of sheep which are herded continuously over a vast area and not lambed in a common facility. It has been suggested that the confinement operation may facilitate disease transmission, reporting or both.

ECONOMIC IMPLICATIONS

The economic impact of scrapie may range from individual producer losses within a marketing area to the loss of export markets for the entire country. The emergence of bovine spongiform encephalopathy (BSE) has made this especially true. The hypothesis that the epidemic of BSE in the United Kingdom may have originated from the feeding of rendered scrapie-infected sheep offal to cattle (75), has put a costly stigma on countries with scrapie. In the past, many nations had simply lived with the disease since it did not have a significant impact on sheep production profits.

Specifically, scrapie has limited the international trade in live sheep, embryos, semen and other ovine products. There is considerable variation on the type and severity of restrictions placed on imports because of scrapie. They range from a total prohibition on imports to simple certification in regard to the absence of clinical signs.

For a more detailed account of import controls and certification, see the section entitled "Preventing the introduction of scrapie into a country".

Another national economic factor is the cost of administering an eradication or control programme. In these cases, funds may be spent on indemnity for those animals ordered to be destroyed, as well as compensation for the producer if the premises must be kept empty for an extended time. Government costs may also include human resources (laboratory personnel and veterinary technical advisers), diagnostic tests and record-keeping.

Traditionally, producer losses were quantified only in terms of deaths from scrapie and a decrease in the value of breeding stock. It follows that losses increase with rising incidence. After the advent of BSE, losses from decreased value of breeding stock have become more extensive, especially when other producers become aware of the occurrence of scrapie in the flock, even at low incidence rates.
Some flocks may exist with an annual scrapie mortality rate of 3-5% (116). Yet, it has been reported that within a flock it is not uncommon to have an incidence of 10-20% (26, 103, 116, 135). It is usually difficult to assess the true incidence of scrapie within a flock unless the disease is notifiable and a high ascertainment rate is attained.

The existence of scrapie and the threat or existence of BSE in some countries has created a new source of financial adversity for the sheep industry. Some countries have imposed bans on the inclusion of ovine, bovine or all ruminant protein into ruminant feeds. Even without statutory bans, disposal costs of sheep offal and waste have increased and these are often passed back to the producer. For example, due to the fear of BSE, in some parts of the United States it is difficult to find a slaughterhouse which will process adult sheep or even lambs on account of the problems involved in disposing of offal. In Great Britain this is not a problem, despite the occurrence of BSE, because the ban is enforced to prevent the feeding of ruminant protein back to ruminants. Sheep offal is allowed to be rendered and recycled only in pig or poultry rations.

AETIOLOGY

Infection and genetic factors

Theories on the cause of scrapie have been debated for many years and the discussion continues today. Initially, arguments over cause centred around a genetic versus infectious origin. The “infectious” theory led to further speculation over the nature of the causal agent. Despite the polarisation of some opinions, it is perhaps acceptable at this point to express the view that scrapie results from the interaction of an infectious agent with the host genome. Different agent strains may interact variably with hosts of different genotypes.

Parry (101) believed that scrapie was not naturally infectious but was a genetic disease caused by an autosomal recessive gene. Data accumulated from 1,400 cases appeared to support his theory. However, he conceded that affected animals harboured a transmissible agent which was infectious by artificial routes.

Evidence for the experimental transmission of scrapie was first reported by Cuillé and Chelle (22) who successfully transmitted the disease from affected to healthy sheep via intraocular injection. Cuillé and Chelle (23) also reported successful experimental transmission to goats and a natural case of goat scrapie was reported in France by Chelle (17). The goat co-habited with scrapie-infected sheep. Subsequently, Pattison and others (105) used goats extensively in experimental studies of scrapie. Chandler (12, 13, 14) added to these discoveries by transmitting scrapie to mice. Later evidence (7, 30, 64) strengthened the argument that scrapie in sheep and goats was a naturally-occurring contagious disease caused by an infectious agent.

A current view is that clinical scrapie in sheep results from the interaction of one or more strains of an infectious agent with one or more host genetic factors. Among the latter, a gene called $Sip$ (Scrapie incubation period) is very important.
Nature of the agent

The various hypotheses for agent structure and the characteristics of the scrapie agent are dealt with fully by Bennett and colleagues in this issue (5). Scrapie infectivity is associated with an abnormal form of a cellular sialoglycoprotein $PrP^C$ coded by the host $PrP$ gene. The transition to the abnormal isoform ($PrP^Sc$) is a post-translational event and results from infection. The presence of $PrP^Sc$ is specific for the diseased state. $PrP^Sc$ is distinguished from the normal isoform by being partially protease resistant. It forms the protein of SAF (see below). Hypotheses of scrapie agent structure include that:

a) $PrP^Sc$ is the agent (prion hypothesis)

b) $PrP^Sc$ is part of the agent coupled to the agent genome (virino hypothesis) and

c) the agent is an unconventional virus with the protein coded by the viral genome.

For a full treatise of the prion hypothesis, the reader is referred to Prusiner and McKinley (111) and Prusiner, Collinge, Powell and Anderton (112) where some of the latest evidence and hypotheses are presented including the concept of Anti-$PrP$ (62).

Research findings indicate that strain differences and mutations can occur in the scrapie agent. Strains can be distinguished by multiple sub-passage in rodents, notably mice. They can be biologically defined by determining incubation period length in mice of different scrapie incubation period ($S_{Inc}$) genotypes and lesion profile by neuropathological examination of the brain. Using this classification method, approximately twenty strains have been identified. The agent can be cloned by serial passage at the highest positive dilution from the latest individual mice to become affected (46).

Other points to note are that more than one strain can be isolated from single cases of natural scrapie. Contrary to popular belief, some transmission attempts from natural cases of sheep scrapie to mice fail. For example, some 19% of attempted transmissions to mice from natural cases of ovine scrapie in Britain were unsuccessful (45). Selection of mutant strains of scrapie can occur following passage across a species barrier; for example, following mouse to hamster passage of strain 139A. This resulted in a permanent change in the scrapie agent genome following reisolation in mice but this is not an invariable consequence of interspecies transmission (80).

Scrapie strains can be divided into either A or C groups. The A group strains interact with $Sip$ genotypes in the standard way (i.e. the incubation period length in the sAsA genotype is short and in the prolonged (pApA) genotype so long that clinical disease usually does not result before the sheep die of other causes or are culled). In contrast, the only C type strain so far identified (CH1641) interacts with the host in the contrary direction.

Finally, it is important to note that the scrapie agent is exceptionally resistant to ultraviolet and ionising radiation (including that used to decontaminate food), physical and chemical treatments. There seems to be little difference in resistance of different strains to chemical procedures, although they do differ in their sensitivity to heat, with wet heat being substantially more effective than dry heat. Samples of 263K-infected hamster brain contained residual infectivity after 1 h at 360°C (9). A single porous load autoclave cycle of 134°C-136°C (30 lbs psi) for 18 min., hold
temperature and time, or six separate cycles of 3 min. each (25) are recommended for hospital instrument sterilisation. This is based on studies on 139A and 22A strains (22A is the most thermostable scrapie strain under autoclaving conditions so far investigated) (79).

Formalin is ineffective for decontamination of the scrapie agent. Chemicals effective or partially effective in destroying infectivity include sodium hypochlorite (2% available chlorine) acting in the absence of organic matter or 1M sodium hydroxide. Taylor provides a full treatise on inactivation procedures (123, 124, 125).

Genetic influences of agent and host

There are two concepts to consider: firstly, genetic variation in the agent and secondly, genetic variation in the host. Both aspects have been covered by Bennett and colleagues in this issue (5). It is convenient to summarise the main features here.

While neither an agent genome nor a nucleic acid component has so far been found, there is evidence for agent strains and strain mutation (80) which is difficult to explain in the absence of a genome. The prion hypothesis (111) has difficulty in explaining these biological features which are claimed to be met by a combined theory recently put forward by Weissmann (131).

In regard to the host, like mice (Sinc gene), sheep have a major gene (Sip) which, with two alleles sA and pA, determines the incubation period of experimental scrapie (31) and of natural scrapie in at least some sheep breeds (39, 67). Under conditions of natural scrapie, Cheviot and Swaledale sheep homozygous for the pA allele do not usually succumb to disease within their normal lifespan. Sheep homozygous for the sA allele usually develop clinical disease in their lifetime.

In some sheep which are sApA, the sA allele may be completely dominant, thus causing the incubation time to be the same as in the sA homozygotes (39). Yet work with experimental scrapie has shown that there is partial dominance of the sA allele resulting in an incubation time somewhere between that for the sAsA and pApA (41, 86). Exposed sA homozygotes, heterozygotes and pA homozygotes may, however, be infected with scrapie and be shedding the agent prior to the onset of clinical signs. This is important in the epidemiology of the disease.

An equivalent gene of Sip has been identified in Argali and Rocky Mountain Big Horn sheep (Maciulis, personal communication). In contrast to sheep, goats appear to be universally susceptible to scrapie (104).

Demonstration of a link between the Sip and PrP genes in Cheviot sheep was demonstrated by Hunter and colleagues (66). Goldmann and colleagues showed that by determination of the gene structure, two alleles of the PrP gene were linked to scrapie in sheep (49) and that different PrP proteins are coded by lines of sheep selected for different alleles of the Sip gene (50). The knowledge of the existence of the PrP gene and means of detection by restriction fragment length polymorphism (RFLP) analysis (49, 67) has provided an explanation of some aspects of the epidemiology of scrapie.

Determination of the Sip gene allelic variation and its frequency in a flock, together with a knowledge of the existing or imported strains of scrapie, may enable a future strategy to be developed to aid in the control of the disease. Also, RFLP analysis could be used as a supplement to other genetic information when developing a breeding
programme within healthy flocks, so that appropriate genotypes within families may be retained and utilised should scrapie subsequently be confirmed in the flock.

RFLP analysis is a relatively simple and rapid procedure. However, agent strain typing using in-bred strains of mice is expensive, takes a long time (perhaps up to ten years) and requires access to a specialist laboratory. It is therefore impractical in the field situation. A successful control programme has been evolved in a large closed flock of commercial Swaledale sheep in Britain using RFLP analysis (65) and this shows promise for successful development on a wider scale.

It should be noted that the Sip gene has been shown to play a role in the control of incubation length. However, there is no evidence that it functions in providing resistance to infection as opposed to development of clinical disease. The role of a naturally-infected asymptomatic carrier has not been determined. These animals could possibly be a source of infection for flock mates. Also, it is possible that different strains and breeds render different outcomes in terms of incubation. Therefore, more research must be completed if this is to be used as a tool to control the disease. The present state of knowledge does not provide an opportunity for employing these techniques to control scrapie in goats or in moufflon.

TRANSMISSION AND EPIDEMIOLOGY

Transmission

Although it is generally accepted that scrapie is an infectious, contagious disease, the means of natural transmission are not understood. To avoid semantic confusion, the following terms will be used as defined below:

a) lateral or horizontal transmission — the spread of infection between unrelated animals via direct or indirect contact at any time or to offspring after parturition;

b) vertical transmission — the spread of infection or genes responsible for disease from parent to offspring via germ plasm at the time of fertilisation or in utero during embryonic and fetal development;

c) maternal transmission — the spread of infection from a dam to her offspring either vertically from female germ plasm, from infection of the embryo, conceptus, placenta or fetus, or laterally, in the immediate post-parturient period.

It has been well established that scrapie can be transmitted laterally between unrelated sheep (7, 30, 64). In a very significant example, Hourrigan and colleagues (64) exposed 140 sheep and goats to natural scrapie, by continual contact with a succession of natural cases of scrapie. The previously unexposed, unrelated animals were introduced to the affected flock at 3 to 9 months of age. Scrapie occurred in 5 of the 140 animals, indicating that scrapie was spread laterally by contact exposure. The animals developed scrapie at 64, 80, 82, 85 and 93 months following initial exposure. Scrapie occurred in 27% of the progeny of these previously unexposed 140 animals. The average age for the development of scrapie in the progeny was 41 months. It was suggested that the higher incidence of scrapie seen in the progeny was due
to exposure at an earlier age. This may indicate that the offspring received a greater
dose of the agent or perhaps had an increased age-related susceptibility. The
investigators did not rule out the possibility that the sheep born at the facility were
exposed vertically.

Evidence of lateral transmission is also displayed by goats contracting the disease
after being reared with scrapie-infected sheep flocks (7, 17, 64, 120).

In order for the disease to be considered contagious, the agent must be shed from
the host in sufficient quantity and in an appropriate way to infect others. Hadlow
and colleagues (58) showed that agent was detected first in lymphoreticular tissue
of the alimentary tract and thus suggested that this is a most likely portal of entry
in natural scrapie. This finding is substantiated by the experimental transmission of
scrapie to sheep and goats by the oral route (106, 108, 109). Other potential routes
of natural infection which have been shown to be effective experimentally are through
scarification (119) and via the conjunctiva (60). Iatrogenic scrapie has occurred in
over 300 sheep of several thousand inoculated with louping ill vaccine. This vaccine
was manufactured using formalised sheep brains contaminated with scrapie agent (51).

The detection of agent in the placenta of Swaledale sheep (108, 109), in
combination with a failure to detect agent in faeces, saliva, urine, colostrum or milk
(57, 58, 64, 106) has led to a fairly wide acceptance that the placenta and perhaps
fetal fluids play a significant role in the spread of scrapie. Hence transmission would
most likely occur from an infected dam to her progeny and other lambs or adults
in close proximity at the time of parturition. Whether this spread would be from direct
contact with the infected tissues/fluid or indirectly from contamination of the
environment is unknown. However, the physico-chemical stability of the scrapie agent
suggests that indirect spread of infection might be as important as direct spread.

There are two problems to consider: firstly, shedding of the agent through the
placenta may not be a constant occurrence. Hadlow and colleagues (58) were unable
to detect the agent in the placenta of two Suffolk ewes affected with scrapie.

Secondly, both Pattison and Hadlow point out that not detecting agent in the
faeces or other excretions or secretions may not necessarily signify absence. It may
suggest inadequate examination (54, 58, 106) or the inadequate sensitivity of the
bioassay system if a heterologous species is used. The method of detection used by
Hadlow involved mouse bioassay. Hence, failure to identify the agent may indicate
true absence or simply represent the decreased sensitivity of the bioassay when crossing
a substantial species barrier. In contrast, the work by Pattison represented sheep to
sheep and sheep to goat transmission.

The agent has been identified by bioassay in intestinal tissue and nasal mucosa
(57, 58) although shedding of infectivity has not been detected through these routes.

Role of the ewe

There is evidence that the progeny of ewes infected with scrapie agent are more
likely to become clinically affected than unrelated offspring of “scrapie-free” dams
or offspring from scrapie-positive sires (30, 64). The expression by the host of the
agent, as covered in the section entitled “Transmission”, is a very plausible explanation
for this occurrence and implicates the ewe as the primary source of infection. This evidence also gives rise to the use of the term "maternal transmission", but precisely how and when transmission occurs (in utero and/or post partum) has not been fully determined.

The phrase "maternal transmission" may sometimes cause confusion among shepherds. In some countries, maternal transmission has a genetic connotation, whereas in others it merely indicates spread from mother to offspring.

In further support of lateral transmission from the dam, data show that the longer offspring have contact with their infected dams post-natally, the more likely they are to develop scrapie (28, 30, 64). Hourrigan and colleagues (64) reported that the incidence of scrapie in lambs removed at birth was 10%, 16% for those removed at four months, 29% when removed at nine months and 41% when removed at twenty months. A similar result was obtained from goats. None of ten goats removed at birth and placed in isolation pens developed scrapie, although counterparts removed at six months had an incidence of 57% and all seven (100%) of those removed at 8 to 10 months contracted scrapie (64).

The questions surrounding the possibility of scrapie spread through vertical transmission (by either germ plasm or in utero) and the potential role of the ewe in this process are still, however, unanswered. Published data, complete with technical and procedural details, are very limited.

In one of the few studies examining the pathogenesis of natural scrapie, Hadlow and associates (58) were unsuccessful in their efforts to detect the agent in five fetuses from infected dams. This particular study was also unsuccessful in finding evidence of scrapie in ovaries or uterine tissue. In addition, the researchers did not find evidence of infection in extraneural tissues from animals less than ten months of age. This work may demonstrate the slow replication of the agent or a decreased sensitivity of bioassays using mice. On the other hand, it may suggest that intrauterine infection does not occur or is a rare event. It is possible that the placenta may act as a type of barrier for the fetus during gestation and releases the agent to the environment during parturition.

Although Hourrigan (63) reported evidence of the scrapie agent in ovary, uterus and one fetus, it is difficult to assess the significance of this work as the technical details have not been published.

Foote and colleagues, of the Utah State University and Scrapie Investigation Center, Mission, Texas (USA), have studied the consequences of reciprocal transfers of embryos between scrapie-inoculated and scrapie-free sheep and goats. Their objective was to determine if embryo transfer (ET) could provide a means of obtaining scrapie-free sheep from infected flocks, hence saving valuable genetic lines. The reluctance to destroy certain bloodlines to control the disease in scrapie-infected flocks often impedes the success of control programmes. If the ET technique is effective, this problem would be overcome. Embryo transfer would be valuable to countries wishing to import new genetic stock from scrapie-affected areas if it could be shown that the risk of transmitting scrapie was at an acceptably low level (37, 38).

In this study, donor ewes were inoculated with scrapie by either the subcutaneous route or the oral route, or both. Zona-intact embryos from the inoculated donors were collected, washed three times and then transferred to recipients with no known
exposure to scrapie. All of the recipient ewes were reported to be free of any evidence of clinical scrapie. They were observed for at least five years post-implantation. A total of 99 offspring resulted from the transfers. Fifty-six of these offspring reached 60 months of age or more, while the remaining 43 died prior to 60 months. Twenty-nine of these originated from donors which developed the disease, but none of the offspring were reported to exhibit signs of scrapie (Foote, personal communication). As of the date of this publication, histopathology results on all offspring are pending.

Foote and associates are also in the process of conducting an embryo transfer project, utilising experimentally-infected goats. This work is in its final year of completion. To date no ET offspring, resulting from scrapie-inoculated does, has shown evidence of scrapie.

The experiment also involved transferring embryos from scrapie-free sheep to scrapie-inoculated recipients and then removing the lambs by caesarian section at term. The intention was to explore the possibility of in utero transmission. Nineteen progeny (nine of which had dams which developed scrapie) from a total of 58 taken by caesarian section reached 60 months of age or more. There was no clinical evidence of scrapie observed in these animals and the microscopic examination of brain tissue is in progress.

Another experiment (42) which examined the risk associated with the transfer of scrapie via the embryo has, however, cast some doubt on the previous assumption that embryos did not play a role in scrapie transmission. Embryos were collected from six donor ewes of the Sip genotype sAsA or sApA which were inoculated subcutaneously with SSBP/1 brain homogenate. The embryos were transferred unwashed into recipient females of the Sip genotype pApA (with the exception of one which was sApA).

All the donor ewes developed clinical scrapie which was confirmed by histopathology. All the recipient ewes remained free of any evidence of scrapie. Twenty-six lambs resulted from the transfers. Six of these died before a year of age from conditions unrelated to scrapie. Six of the remaining twenty offspring (all Sip sAsA) were confirmed to have scrapie at approximately two years of age. The remaining animals (eleven sApA and three pApA) are healthy at the time of writing.

The authors suggest that the infected donor ewes passed scrapie infection to their ET progeny via the embryo. They did not rule out the possibility that the pApA recipients may have harboured a subclinical infection and transferred the disease in utero (42). If the histopathological results of Foote and colleagues support the clinical absence of scrapie in the ET offspring, further work should be conducted to determine what factors may have made the difference in allowing disease transmission in one of the studies but not the other.

Both of the projects on embryo transfer involve experimental scrapie which may not be totally indicative of the natural situation. Foote’s group is in the process of repeating their work using field cases of scrapie. Eight of the ET offspring from naturally-infected donors are now four years old and three ET offspring are two years old. None of the animals have displayed evidence of scrapie (Foote, personal communication).

All the above information clearly demonstrates the high risk associated with offspring of a scrapie-infected dam. However, unrelated lambs will also be at risk
if they have close association with an infected ewe. The same study (64), which showed that the progeny of scrapie-affected dams have a greater incidence of scrapie, also showed that 25% of the progeny from unaffected sires and dams developed scrapie. It has been noted in the United States that unrelated lambs kept in the same "mothering up" pen as a scrapie-infected ewe show a high likelihood of developing the disease (Detwiler, unpublished observations). Failure of the USA Bloodline Control Program can, for the most part, be attributed to lateral transmission between unrelated sheep.

If there is subclinical shedding of the agent by silent carriers, it is theoretically possible that some of the unaffected dams may have been of the $Sip$ genotype which produces a long incubation and have infected their offspring. In Great Britain, veterinary administered flock control schemes include measures to prevent lateral transmission, such as those described above, with severe culling of appropriate bloodlines to reduce the risk of transmission.

**Role of the ram**

The ram is thought to play much less of a role in the spread of scrapie infectivity than does the ewe. The failure to detect the agent in testes and seminal vesicles or semen may indicate a lack of infectivity in semen or, if present, at a level undetectable by mouse bioassay (58, 63). With semen, negative assays across a species barrier, even by inoculation, are less convincing than assay within the species, which also has the benefit of increasing sensitivity due to growth and development of the offspring over many years. In the United States, Professor Foote and colleagues are nearing the conclusion of a project involving artificial insemination of semen from rams experimentally inoculated with scrapie. Their results so far are consistent with a low risk of paternal transmission (Foote, personal communication). However, the design of this project has not taken full account of the genetic variability of the sheep in respect of the $Sip$ gene and evaluates only experimental scrapie introduced by an unnatural route. Also, the number of progeny is very small. The pathogenesis of the natural disease may be different in regard to infection of the male reproductive tract.

Most flock management practices do not allow the ram continuous contact with the ewes or their lambs outside the breeding season. This minimal contact would reduce horizontal spread from any source of infection in the ram. Thus, the main role of the ram seems to be in determining the $Sip$ genotype of his offspring though the possibility for transmission via semen cannot be ruled out entirely.

**Role of the environment**

The extent to which scrapie is transmitted by a contaminated environment, including pens, barns, feed, water, bedding and other fomites is unknown. The remarkable resistance of the agent to inactivation leads one to believe that it may survive in the environment for a number of years. This is supported by the results of an experiment (10), in which an homogenate of hamster brains from terminally-affected animals previously inoculated with hamster-passaged 263K strain of scrapie was mixed with soil and buried. Three years later, the contaminated soil still contained some residual infectivity though there was a loss of between 2 and 3 log units or reduction by over 98% of the infectious units during the period of interment. When this loss occurred is unknown (i.e. just after burial, during the period of interment or just before exhumation). There was very little leaching into deeper soil layers. This
indicates the long-term potential for the agent to survive in the soil. However, the epidemiological significance would depend on the amount of contaminated soil that animals might consume, the possibility of absorption by pasture and nematodes or other vectors which the host might also consume.

Infectivity has been detected in nematodes (64) but it is not known whether they would have been capable of transmitting disease to sheep. However, experimental studies with *Haemonchus contortus* in sheep and goats and *Nippostrongylus brasiliensis* in rats and *Syphacia obveleta* in mice were unsuccessful (36).

In Iceland, pastures which had been grazed by scrapie-affected sheep were left vacant for several years before restocking with sheep from flocks believed to be scrapie-free. Some of these new sheep developed scrapie (99). As this was not a controlled experiment, it is difficult to draw firm conclusions from this observation.

The epidemiology of scrapie resembles an unfinished puzzle. There is a general outline of the picture, but many details are missing. In addition to the questions posed above, other unknown factors regarding the transmission of scrapie between infected species and individuals are:

- *a*) what is the infective agent dose for sheep and goats?
- *b*) what is (are) the route(s) of exposure in natural scrapie?
- *c*) at what point in the incubation period does the host shed the agent?
- *d*) is shedding continuous or intermittent? and
- *e*) what are the quantitative aspects of shedding?

Without a better understanding of natural transmission it will be very difficult to eradicate scrapie from a country with an endemic problem.

**Epidemiology of scrapie within a flock**

Scrapie occurs most frequently in sheep of either sex between two and five years of age (26, 116). The modal age of clinical onset is about three and a half years (100). In the United States, between 1947 and 30 September 1991, 82% of the 779 sheep diagnosed with scrapie and of known age (51 additional cases had no age listed) were between two and five years old. Approximately 70% were between two and four years of age (USDA/APHIS, Veterinary Services, unpublished statistics). The United Kingdom surveillance database, Veterinary Investigation Diagnosis Analysis (VIDA), records the majority of scrapie cases to be between two and four years old (134).

Cases of the disease are rare before 18 months of age (29, 116; USDA/APHIS, Veterinary Services, unpublished statistics). However, a few cases of natural scrapie have been reported in sheep as early as 7 months (in Iceland) (116) and at 10-12 months of age (69, 70). Histological evidence of natural scrapie in apparently normal sheep has been reported in lambs 11 months old (27). Since it is thought in general that most sheep which are exposed and eventually succumb to the disease are infected at birth or shortly thereafter, the age at onset of clinical signs will reflect the incubation period.

Several observers have noted that once scrapie becomes endemic in a flock, age at death tends to decrease over time. The initial cases will usually be in four-
five-year-old sheep. The age of occurrence then declines progressively to 18-24 months: in Britain (40, 71), in France (15, 16), in Iceland (116) and in the USA (Detwiler, unpublished observations). Foster and Dickinson (40) state that the most plausible reason for their observations is an increase in exposure to the agent.

The incidence of clinical scrapie within a flock is variable. Some flocks may only experience a 3-5% annual mortality (116), whereas reports of annual losses from 10% to 20% are not uncommon (103, 116, 135). One commercial flock in Iceland had an annual mortality of 50% (116).

As alluded to in the section entitled “Current occurrence of scrapie within countries”, with no diagnostic test for infection in live sheep, the existence of scrapie often depends on the ability of the owner to recognise the disease and his willingness to have it diagnosed. It can be difficult to obtain the true incidence of scrapie within a flock, especially when economic factors influence reporting. On many occasions, the incidence is higher than the limited data indicate (26; Detwiler, unpublished observations).

**Epidemiology of scrapie in goats and moufflon**

Most reported occurrences of natural scrapie in goats have been associated with the presence of scrapie in sheep. Indeed, many of the affected goats had been reared with the sheep (7, 17, 64, 120, 128). All four cases of natural scrapie in goats in the United States have been sheep-associated (USDA/APHIS, Veterinary Services, unpublished statistics).

However, Hourrigan and colleagues (64) observed that scrapie could be spread from goat to goat with no sheep contact. Andrews and colleagues (2) reported similarly in the United Kingdom that transmission in one outbreak was unlikely to be via exposure to infected sheep. While not proven, an alternative source might have been infected proprietary feed. In the United Kingdom, six cases of scrapie have been confirmed in two different flocks of moufflon. The disease appeared to be endemic in both of the flocks and it has not been possible to determine the origin of the disease in either case. One flock was kept apart from domestic sheep although there were animal movements in and out of the flock. The second flock was maintained with a Soay sheep flock. However, scrapie has not been recorded in that Soay flock. The first confirmed moufflon in the second flock was an import from Belgium. The clinical signs observed were identical to those observed in domesticated sheep (133).

**CLINICAL SIGNS**

Scrapie is a non-febrile, insidious disease in sheep and goats. Due to the damage to nerve cells, affected animals will usually show behavioural changes, tremor (especially of head and neck), pruritus and locomotor incoordination which progresses to recumbency and death. The clinical course of scrapie is usually of significant duration (one to six months). However, there has been one report of a case with only a two-week duration (69).

The onset of clinical signs is often marked by a slight change in behaviour. For example, an animal may become more nervous or aggressive, and may separate itself
from the rest of the flock. In many instances, these subtle changes may pass unnoticed. Some sheep appear to be demented, or exhibit head pressing, or “star gazing”.

Hypersensitivity is another characteristic of scrapie. An affected animal may appear normal if left undisturbed, but when handled, tremor may become excessive and the animal may even fall in a convulsive-like state. Scrapie-affected sheep, but not goats, have a tendency to lose much weight, despite retaining a normal appetite.

Scrapie acquired its name from the characteristic sign of “scrapping” or rubbing against fixed objects. Pruritus may be so subtle as to go undetected or can be so dramatic that an animal will rub off most of its wool (Fig. 1). The areas of wool loss may sometimes be rubbed raw. Some sheep will pull wool from their sides or bite at their legs. Affected goats are less likely to rub against fixed objects, but scratch vigorously with hind feet and horns (Hadlow, personal communication). Some sheep may exhibit a “nibble reflex” when rubbing themselves or when scratched by hand.

Motor abnormalities often include a high-stepping (trotting) gait of the forelimbs and a “bunny hop” movement of the back legs. This gait is especially noticeable when the animal is made to run. As the disease progresses, there can be severe ataxia of the hind limbs causing the animal to sway, support its hindquarters against a fence when standing and have difficulty rising.
Not all animals exhibit all signs of the disease. For example, an animal showing severe pruritus may show little if any incoordination, and vice versa. There may also be differences between breeds.

In a letter to the editor, Clark (18) stated that the Scottish Agricultural College Veterinary Investigation Centre (Thurso), found that 21% of sheep “found dead” and submitted for diagnosis were confirmed by histopathological examination to have scrapie. While sudden death may be an actual occurrence of scrapie, it is more likely that shepherds missed the progression of clinical signs.

Clinical signs may differ between countries. In Great Britain, debility and weight loss are not always seen (26, 73). Body condition often remains good and some sheep may actually gain weight (73).

Wasting and debility in sheep with scrapie are common in the United States and in the Himalayan foothills (139). However, in the Himalayan foothills pruritus was the predominant sign, whereas in the United States pruritus is very often subtle, if observed at all. Signs distinctive of scrapie in the United States are tremor and incoordination.

Observers in Iceland reported a change in the clinical picture of scrapie over the years. Prior to 1968, the disease manifested itself as tremor and ataxia. Subsequently, pruritus was more commonly seen and often became the predominant sign (116).

In moufflon, clinical signs were indistinguishable from sheep scrapie (133).

PATHOGENESIS AND PATHOLOGY

Pathogenesis

A significant amount of work has been conducted to investigate the pathogenesis of scrapie, using a wide variety of experimental models of scrapie in mice and hamsters. The studies have revealed substantial information on the mechanisms of pathogenesis and on the control of the incubation period. However, little has been done using sheep and goats, the natural hosts. There is even less information available on the pathogenesis of natural sheep scrapie in breeds other than the Suffolk. This is because such studies are time-consuming and extremely expensive. Since in natural scrapie specific morphological lesions occur only in the central nervous system, around the time of onset of clinical signs and thereafter, studies on pathogenesis during incubation have centred on determining the infectivity of different tissues by bioassay during and after the incubation period. As cell culture methods are not available to assay the scrapie agent, bioassays in laboratory rodents are the only cost-effective alternative. Bioassays across a species barrier usually reduce their sensitivity considerably. However this has been less of a problem when titrating the infectivity in tissues from naturally-infected Suffolk sheep and goats in strains of susceptible mice.

The absence of a test to identify which animals are naturally infected with scrapie before the onset of clinical signs creates a problem of selecting infected animals for study, with the risk of choosing one or more animals which are not even infected (54). Animals for study are thus selected on the basis of a complete knowledge of the history of the flock and, in particular, female lines within it which have a consistent
record of disease occurrence. Clearly this is at best unlikely to be 100% effective and could give misleading data about negative transmission in certain circumstances.

Until the epidemiology of scrapie in sheep and goats is fully understood, difficulties will persist in the prevention and control of the disease. At this time uncertainties prevail about the route and timing of exposure, the source of the agent, the infective dose and the role that preclinical or subclinical carriers may play in the evolution, transmission and perpetuation of the disease in a flock.

Pathogenesis of experimental scrapie in laboratory rodents

Studies of experimental mouse scrapie have produced results which are broadly similar to those found in sheep and goats (34). However, more detailed and sensitive bioassay studies have been possible in models of scrapie in both hamsters and mice.

It is believed that in order for clinical scrapie to develop, the agent must invade and replicate in certain target areas of the CNS (78). The examination of scrapie pathogenesis following infection via peripheral routes (intraperitoneal, intravenous, subcutaneous, intra-gastric) reveals that the agent replicates in certain organs of the lymphoreticular system (LRS) prior to invading the CNS. The spleen appears to play an important role, as its absence will prolong incubation (44). Lymph nodes, especially those associated with the viscera, are also sites of agent replication (72).

The cells of the LRS which support scrapie replication have not yet been identified. Certain observations have indicated that the cells of the lymph nodes and spleen which are metabolically and structurally stable are more likely to be replication sites (72).

The thoracic cord seems to be the first site of CNS invasion following peripheral infection. It is strongly indicated that the agent moves from the LRS to the thoracic spinal cord probably via sympathetic nerve fibres. From here, the infection progresses along the cord in both directions (72, 76, 77, 78). Once the agent invades the CNS it has been suggested that infection spreads intra-axonally, with the rate being approximately 1 mm/day (77).

Within the CNS there is a direct relationship between areas of agent replication and the development of lesions. Initially, the grey matter vacuolation pattern models the progression of infectivity. Later, as the disease advances to its clinical stages, the lesion profile reflects the characteristics of the certain scrapie and mouse strains used (72).

Certain restrictions placed on the process of agent replication in different tissues are apparently the cause of the slow course of scrapie infection within a host (32). There are a limited number of replication sites in a limited number of non-replaceable cells. The Sinc gene is thought to regulate the rate of spread by controlling the cell-to-cell movement of the agent and/or its intracellular multiplication. The Sinc gene appears to exercise this control at the LRS/nervous system interface and within the peripheral and central nervous systems (74).

Studies of natural and experimental infections in sheep and goats have not always corroborated these findings, but neither have they produced any contradictory results (55, 58).
Pathogenesis of scrapie in sheep and goats

Hadlow and colleagues (56, 58) have examined the temporal distribution of scrapie infectivity in preclinical, clinically-normal and clinically-affected Suffolk sheep of various ages, from high-risk families of an experimental, fully-recorded flock with natural scrapie. They have also studied tissues from Suffolk and some other breeds at the clinical stage (56). Infectivity titres were measured in susceptible random bred Swiss mice which were inoculated intracerebrally (58). Infectivity was not detected in tissues from six Suffolk lambs euthanised immediately after birth, two 3-month-old lambs, or eight lambs aged 7 to 8 months, all from high-risk families.

Infectivity was first detected preclinically in tissues from eight of fifteen lambs of the same origin which were 10 to 14 months old. The agent was consistently demonstrated in the retropharyngeal and mesenteric-portal lymph nodes and spleen. It was also found occasionally in tonsil, prescapular and prefemoral lymph node, ileum and proximal colon. The discovery of the agent in tonsil, retropharyngeal lymph node and intestines suggests that it is introduced into the host orally (58).

Tissues from three 25-month-old clinically-normal Suffolk sheep were also inoculated into mice. Only one of these sheep was shown to be infected. Its dam and that of another ewe died of scrapie. The agent was widely distributed in lymphatic tissues, ileum and proximal colon and was also detected in the CNS in limited areas (medulla oblongata and diencephalon), at low titres. There was no evidence of the agent in the cerebral cortex, midbrain or cervical spinal cord and histopathological changes were not observed (58).

Hadlow and colleagues (58) also studied the distribution of the agent in tissues of clinically-ill, naturally-infected Suffolk sheep. Microscopic examination of brain tissue from all nine sheep revealed lesions characteristic of scrapie. The bioassay results showed the agent to be widely dispersed throughout central nervous tissue. Infectivity titres in the central nervous system were much higher than in non-neural tissues. In most sheep, the highest titres were found in the diencephalon, midbrain, medulla oblongata, cerebellar cortex and spinal cord. These correlated well with the areas of brain showing the most severe histological changes. The agent was also found at lower titres in spleen, tonsil, various carcass lymph nodes, proximal colon and distal ileum.

Small amounts of infectivity were found in nasal mucosa, distal colon, cerebrospinal fluid, pituitary gland, sciatic nerve and adrenal gland. Barely detectable amounts of agent were present in the pancreas, liver, bone marrow, thymus and the supramammary lymph nodes. None of the sheep had detectable infectivity in the following tissues: blood clot, fetus, heart, kidney, lung, mammary gland, skeletal muscle, ovary, placenta, saliva, salivary glands, seminal vesicle, testes, thyroid or uterus.

Included in the other breeds examined was a 56-month-old Montadale ewe with clinical and histological evidence of scrapie (56). This animal showed no evidence of agent in non-neural tissues, although it was widely dispersed throughout the brain but in moderate titres. Small amounts were found in the spinal cord. In two Cheviot ewes, aged 48 and 81 months, the agent was present in the suprpharyngeal lymph node, and in one of them, in the tonsil and ileum. These were the only non-neural tissues from Cheviots with infectivity. The 81-month-old ewe had moderate titres in
all parts of the CNS. The other animal had extremely low titres in the CNS and in view of the findings in Suffolk sheep, the authors considered that clinical disease would not have been expected from these findings (56).

The non-neural distribution of the agent in a Targhee and a Rambouillet was similar to that in Suffolk sheep. In the central nervous system of the Targhee, infectivity was limited to the diencephalon, medulla oblongata, midbrain and spinal cord. In the Rambouillet, agent was present in the corpus striatum, diencephalon, midbrain, medulla oblongata, cerebellum, spinal cord and also in the sciatic nerve (56). It is very difficult to draw conclusions about differences between breeds with only one or two sheep examined from each of the breeds studied. If the predominant strain of agent is not the same in different breeds, variation in the results of bioassays across the species barrier would be expected (72).

Renwick and Zlotnik (113) demonstrated infectivity in brain tissue from an 18-week-old clinically-normal Border Leicester lamb born to a ewe clinically-affected with scrapie; this was subsequently confirmed by histopathology. Hourrigan (63) reported finding infectivity by mouse inoculation in extraneural tissues of an apparently normal lamb, as young as four months old.

These reports may simply be solitary incidents, demonstrating one extreme of the scrapie infection spectrum; for even when Hadlow and colleagues (59) inoculated fetuses in utero, the earliest that they were able to detect the agent was 180 days post partum. However, due to the fact that the findings of Renwick and Zlotnik and Hourrigan do not concur with the more comprehensive studies of Hadlow and colleagues (56, 58, 59), further investigations should be carried out to determine their significance.

From these studies it is possible to develop a tentative hypothesis of the broad outlines of the pathogenesis of scrapie in Suffolk sheep, namely that infection probably results from oral exposure. Following exposure, a silent or zero phase ensues when no infectivity can be detected in any organs. This lasts for over eight months. After this period, the agent replicates in intestinal and lymphoreticular tissues which include spleen, retropharyngeal and mesenteric lymph nodes. The agent spreads to most lymph nodes but apparently not to many other non-neural tissues and, when it does, only low titres are experienced. Replication continues in the LRS for about two years before it can be found in the brain. The means by which the agent moves from the LRS to the brain has not been determined in sheep but haematogenous and/or neural spread have both been suggested. Once in the CNS, the agent continues to replicate to high titres. In Suffolk sheep and some other breeds the agent remains in the extraneural tissues throughout the infection (56, 58) but in the single Montadale case studied, infectivity was not found outside the brain. Whether it was never there or was eliminated is unknown but exploitation of this phenomenon could have a potential value if scrapie cannot be eliminated entirely.

A study of naturally-infected goats at the clinical stage of the disease (57) gave results similar to those found in clinically-affected Suffolk sheep. In particular, no infectivity was detected in blood clot, bone marrow, faeces, kidney, mammary gland, milk, skeletal muscle, ovary, salivary gland, serum or uterus.

**Differences in tissue distribution between sheep and goats and laboratory rodents**

In contrast to studies of sheep and goat scrapie, the scrapie agent has been found repeatedly in the salivary glands of infected mice (34). This prompted the suggestion
that saliva may play a role in the lateral transmission of scrapie in mice (98). Nevertheless, transmission between mice seems not to be a hazard during experimental scrapie studies, whether from salivary or any other source. There has only been one report of isolation of the agent in the salivary gland of experimentally-infected goats (107) and the agent has never been found in saliva from naturally-infected sheep and goats (57, 58, 64).

The scrapie agent has been detected in low levels in concentrated extracts of blood from infected hamsters (33). Except for the initial few hours after infection, the agent has not been identified in mouse blood (33, 34). Numerous attempts to identify the agent in whole blood and serum from sheep and goats have been unsuccessful (57, 58, 107).

It is important to note that although the agent was not detected in certain tissues, secretions, or excretions of sheep and goats, this does not necessarily mean that it is absent. It may exist in such low amounts that current methods of detection are inadequate, especially when conducting bioassays across the species barrier.

Pathology

The pathological changes of scrapie in sheep and goats are confined to microscopic changes in the CNS (53, 136). The lesions are characteristically found in the grey matter of the brain stem. They include neuronal vacuolation, other forms of neuronal degeneration including some cell loss, astrocytosis, and generalised vacuolar or spongiform lesions of the grey matter neuropil. The majority of vacuoles are intraneural either in the perikaryon or in neurites but some may be paraneural, perineural or not associated with perikarya at all. Vacuolation is most often found in the medulla, pons, midbrain, and thalamus (93). In the moufflon, distinct lesions are also found in the cerebral cortex (133).

Usually a most prominent change is cytoplasmic vacuolation of the neurons in the medulla, pons and mesencephalon. The nucleus is often displaced to the cell periphery by large, single or multilocular vacuoles. On occasions, the vacuoles may contain eosinophilic fibrillar, globular or finely granular material (43).

Neuronal vacuolation may exist in apparently healthy sheep. However, the number of vacuoles is very small and they exist without the other pathological changes of scrapie (43, 116, 137, 138).

Other degenerative changes of neurons exist throughout the midbrain and include chromatolysis, pyknosis and sclerosis. Neurons often appear shrunken and angular. There is usually an increased basophilia of the cell body (93).

The above lesions are accompanied by a glial cell proliferation or hypertrophy usually affecting astrocytes. The astrocytosis may be demonstrated by Cajal’s stain or by immunocytochemical staining for glial fibrillar acid protein (GFAP). Information of astrocytosis is of limited diagnostic value as it is non-specifically associated with many other infections and insults to the CNS (43, 93). Nevertheless, it is of value in experimental studies in mice because other causes are most unlikely.

The presence of amyloid plaques is common in mice and hamsters infected with some scrapie strains (43); Beck and colleagues (3) reported them to be rare in the natural disease. Gilmour and colleagues (48) reported cerebrovascular amyloidosis in 11 of 20 sheep of six different breeds with natural scrapie. The presence or absence
of amyloid plaques may result from the action of different strains of agent. Amyloid plaques can be confirmed by staining with Congo red and examining under polarised light for apple green birefringence. Scrapie-specific plaques also immunoreact for PrP\textsubscript{Sc} (84).

The pathological changes of scrapie do not occur to the same degree in all breeds of sheep. It has been proposed that the breed of sheep and the strain of agent are among factors which may influence the severity and distribution of these lesions (43, 54). In addition, it has been noted that the severity of clinical signs does not always correlate with the severity of the pathological changes in the brain (58).

**DIAGNOSIS**

There are several methods which may be used to diagnose scrapie in sheep and goats. At present, however, they depend on the occurrence of clinical signs in combination with histopathological confirmation. In addition, one or another of the following methods provides supporting evidence for a diagnosis of scrapie:

- immunohistochemical detection of PrP\textsubscript{Sc} in paraffin sections of the CNS
- immunoblotting for PrP\textsubscript{Sc}
- the detection of SAF in brain extracts by electron microscopy.

Transmissibility of the disease (e.g. by mouse bioassay) is a sound but impractical method of disease confirmation due to the long incubation period for positive cases and the potential inaccuracy of a negative result. However, it may have value in confirming the first introduction of scrapie into indigenous sheep in a country.

**Histopathology and immunohistochemical detection**

**Histopathology**

For a full examination, sections of the medulla, pons, cerebrum, midbrain, thalamus, cerebellum and anterior spinal cord should be examined, although findings are most prominent in the medulla, pons, midbrain and thalamus. The lesions characteristically found in scrapie are:

- neuronal vacuolation
- neuronal degeneration and loss
- vacuolation of grey matter neuropil
- astrocytosis
- occurrence of amyloid plaques (sometimes).

There is evidence that not all breeds of sheep exhibit the same severity or distribution of lesions making it more difficult to obtain a definitive diagnosis in some cases (43, 54). It should be noted that brain tissue from apparently normal sheep may display occasional vacuolated neurons. However, when present, these are few in number and are not accompanied by the other characteristic changes (43, 116).
Immunohistochemical detection

Polyclonal antibodies have been used to stain formalin-fixed, paraffin-embedded sections from brains of animals and humans with spongiform encephalopathy. As stated above, the finding of amyloid plaques in natural scrapie by conventional staining methods is inconsistent. PrP\textsuperscript{Sc}-specific antisera raised against purified preparations have been shown to stain thin sections of the sub-ependymal and sub-pial regions of scrapie-infected hamster brains, but not normal brain material. These sections also stain with Congo red dye and show green-red birefringence under polarised light indicating that they represent amyloid plaques composed of extracellular filaments (4, 24, 81, 82, 110).

Detection of SAF

The detection of SAF is one of the criteria by which a disease can be established as a prion disease. Using negative stain electron microscopy, SAF have been consistently detected in detergent-treated extracts of brain from experimentally-infected mice (90), as well as from experimentally- and naturally-infected sheep (47, 114, 118) (Fig. 2). SAF have not been identified in brain extracts from normal rodents or sheep, or from rodents affected by conventional viruses or chemical insults (92). SAF have also been found in non-neural tissues including spleens of affected sheep (114) (Fig. 3).

SAF have been found in preclinically-infected hamsters and mice (91). Current methods have not yet proved to be useful to detect SAF in preclinical sheep. The practical problem is one of sensitivity. At present, the detection of SAF indicates that an animal has the scrapie-associated protein present and this can probably be attributed to infection with scrapie, but the absence of SAF does not necessarily mean that the animal is free from scrapie infection.

Brain tissue submitted for SAF detection must not be fixed but should be frozen, ideally at $-70^\circ$C. At that temperature, the tissue can be kept indefinitely. Although SAFs are resistant to autolytic change, samples should be transported to the diagnostic laboratory in the frozen state.

Immunoblotting techniques for scrapie-specific protein

Immunoblotting techniques such as western blot, dot blot and slot blot analysis have been used to detect the presence of the scrapie-specific protease resistant protein, PrP\textsuperscript{Sc}, in the brains and spleen of scrapie-infected animals (Fig. 4). Prior to immunoblotting, PrP\textsuperscript{Sc} is extracted using detergent and purified by differential centrifugation and enzyme digestions. Immunoblotting of this material using polyclonal antibodies raised in rabbits against mouse or sheep PrP\textsuperscript{Sc} demonstrates the existence of this scrapie-specific protein in clinically-infected sheep (114). There are also indications that these methods may be successful using lymphoid tissue in preclinically affected sheep (68). Due to current limits in the sensitivity of detection, the absence of the protein does not equate with absence of infection. There were some promising, but nevertheless inconsistent, results in this study so further work is required before it can be routinely used in field incidents of suspect scrapie.

There is an ongoing project in the United States to develop this method and use it as a practical diagnostic tool. Blind trials between four laboratories have given encouraging results.
FIG. 2

Electron micrographs of negatively-stained, purified SAF from brains affected with
(a) natural sheep scrapie;
(b) experimentally-transmitted sheep scrapie; and
(c) mouse scrapie strain ME7 (2% uranyl acetate stain)
Micrographs reproduced with the kind permission of R. Rubenstein (114)
Electron micrographs of negatively-stained SAF from spleens of
(a) sheep with experimentally-transmitted scrapie; and
(b) mice affected with scrapie strain ME7
(3% phosphotungstic acid, pH 7.2)
Micrographs reproduced with the kind permission of R. Rubenstein (114)

In limited studies, immunoblotting techniques have been shown to be useful in
detecting $\PrP^{Sc}$ in autolysed mouse brains five days post mortem (Rubenstein, personal communication). This would be useful to support a diagnosis of scrapie in
sheep where there is a delay in proper sample collection, and autolytic change may
not permit diagnosis by histopathology. If this method is contemplated, then
appropriate samples must be frozen for such studies.

Differential diagnosis

The clinical signs of many cases of scrapie are quite distinct and can be easily
recognised (see chapter entitled "Clinical signs"). They include behavioural changes,
Scrapie-affected brain tissue from scrapie agent 263K-affected hamsters (263K), scrapie agent ME7-affected mice (ME7), natural (NAT), and experimentally-transmitted (EXP) cases of sheep scrapie was processed for purification of PrPs. Normal brain tissue from hamsters (NH), mice (NM) and sheep (NS) was processed similarly. Samples were electrophoresed on 15% polyacrylamide gels and either silver stained or analysed by western blots. For EXP-PK, the purified SAF sample was treated with proteinase K (50 µg/ml for 30 min at 37°C) before electrophoresis.

tremor, pruritus and incoordination, progressing to recumbency and death. However, in the early stages of the disease, there are several other conditions which could be confused with scrapie including:

- **ectoparasites** (lice and mites) – can be eliminated by parasitological examination;
— pseudorabies (Aujeszky’s disease) — can be ruled out by an extremely short clinical course in ruminants (36-48 h) and the finding of a high fever;

— rabies — not a problem in rabies-free countries, but it should be considered in rabies endemic areas if the clinical course of the suspect has been shorter than ten days; because of the human health risk, rabies should be investigated in a specialist laboratory by brain examination in all animals dying in this period in a rabies-affected region;

— listeriosis — a febrile condition but usually a short clinical course in sheep and goats may eliminate this possibility; often associated with silage feeding; circling is a common feature;

— ovine progressive pneumonia (maedi-visna) — can be ruled out by a serological testing;

— pregnancy toxaemia (ovine ketosis) — is a seasonal disease of malnourished pregnant ewes and can be diagnosed by serum analysis;

— chemical and plant toxins — may also be difficult to eliminate on ante-mortem inspection if no source of toxin can be positively identified; liver function tests and specific tests (e.g. for lead or organophosphorous toxicity) may be helpful;

— hypomagnesaemia — short clinical course and may be diagnosed by plasma magnesium levels.

PREVENTION

Routine methods of preventing infectious diseases which are laterally transmitted are vaccination, testing and removal, quarantine, and/or imposition of movement restrictions on animals and animal products. Since the scrapie agent elicits no detectable immune or inflammatory response in the host, the use of vaccines and serological tests for antibodies are impossible at present. Current diagnostic tests for sheep and goats are not applicable to live animals under farm conditions. This prohibits the identification of animals which are incubating the disease or carrying the agent. Such animals could be shedding the agent, thus infecting other individuals before the onset of clinical signs in the carrier. Therefore, the only absolute way to prevent the introduction of scrapie is to prohibit all movements of sheep and goats and their products into a free country, region or flock. However, by taking certain precautions, the risks of introduction can be reduced without having to implement a total ban on movement.

Preventing the introduction of scrapie into a country

The only risk-free approach to preventing scrapie from being introduced into a country free from the disease is to prohibit the entry of all live sheep and goats, germ plasm and other ovine/caprine products, and ruminant-derived protein for animal feed or to import from scrapie-free countries. At present, the only countries which are generally accepted as being free are Australia and New Zealand. Although other countries may be scrapie-free, the lack of a preclinical test makes it difficult to confirm and usually there is inadequate surveillance in all constituent flocks to justify the
claim. Also, even in free countries, if scrapie was ever introduced, it may take five years or more before it was clinically recognised due to the long incubation period; hence the importance of strict quarantine measures in these countries.

For many countries, it is not feasible to prohibit all importations of live sheep and germ plasm as the introduction of new genes is essential to the advancement of the domestic and export industry. The only approach to establishing the absence of infection is to monitor the imported animals and their offspring over an extended period of time.

Another way of reducing the potential risk is to limit the importation of animals to rams only. As discussed in the sub-section entitled “The role of the ram”, the ram appears to play much less of a role in the transmission of scrapie. Work in progress by Foote and colleagues (38) may conclude that under certain conditions, embryo transfer and artificial insemination substantially reduce the risk of introducing scrapie into a country or flock to an acceptable level.

A country with endemic scrapie may still place restrictions on the importation of sheep from other affected countries. Such restrictions are usually prompted by the concern about introducing a new “strain” of scrapie into the country which might interact with existing sheep genotypes in an unexpected way.

A review of the import requirements for sheep and goats from a number of countries disclosed:

1. The minimum mandate by most countries is some type of certification about flock and individual animal history in regard to scrapie. Many require that “the sheep or goats have not been affected with or exposed to scrapie, or originated from, or have been on any premises which were infected or source flock premises [a source flock is a flock presumed to have been a source of scrapie but in which disease has not been confirmed] in the last.... months/years” (this certification may vary between six months and ten years). “The animals are not the progeny of, nor related to any animal found to be affected with scrapie, nor removed from a premises located in an area quarantined for scrapie.”

2. Some countries will allow entry of live animals but require an extended post-entry quarantine of up to five to seven years. Others will allow entry of live animals into quarantine but only release the progeny after a prolonged period of time.

3. There is some allowance for the importation of embryos which are collected in accordance with stringent guidelines, such as those recommended by the International Embryo Transfer Society. Embryos may be allowed entry into a country with or without post-entry quarantine requirements. In some cases, post-mortem examination is required for the donor and recipient animals.

4. The importation of sheep, goats, and their germ plasm is prohibited because of the existence of scrapie in the exporting country.

The emergence of BSE in the United Kingdom has revealed a new potential vehicle for exposure of sheep and goats to scrapie, namely, through the feeding of animal proteins (meat-and-bone meal) made from rendered scrapie-infected carcasses. With this in mind, countries should monitor the animal protein which is incorporated into rations for sheep and goats and, if warranted, may introduce effective legislation to prevent the feeding of all ruminant protein or that from the highest-risk offals to ruminants.
Preventing the introduction of scrapie into a flock

In countries where scrapie is endemic, the ideal means for preventing the introduction of the disease is to maintain a closed flock. If animals must be added, all replacements should originate from "scrapie-free" flocks. In reality, this may be virtually impossible to do, as there is no test available to ensure freedom from infection in purchased stock. The buyer has to rely heavily on the integrity and honesty of the seller, but because of the long incubation period, the seller may not be aware of the problem, particularly if a closed-flock policy is not maintained. Provided that an adequate monitoring system is in place, clearly the longer the period of freedom from disease, the more meaningful is any certification of freedom supplied.

The absence of a diagnostic test leaves few protective options for the purchaser. Listed below are some guidelines which may help to reduce the risk of introducing scrapie when purchasing sheep:

1. Ask the owner and, with his/her agreement, his/her veterinarian, if there have been any cases of scrapie, or any other neurological or "scratching" disease which may or may not have been confirmed by laboratory procedures and determine the policy for laboratory examination of such diseases.

2. Check flock records to see if there have been any unexplained adult deaths, and establish the policy in regard to new introductions.

3. If the country has a control programme, check to see if there have been infected animals or tracebacks to the flock.

4. If there is a national scrapie control scheme, check that the seller is a member and that he is compliant.

5. If scrapie is predominantly confined to one or two breeds in a country, the purchaser may wish to avoid buying animals of those breeds.

6. Introduce new genes through rams and not ewes.

7. If ewes are brought into the flock, have them lamb in a separate facility if at all possible.

These guidelines may help to reduce the risk of introducing scrapie into a flock but they should not be considered as offering absolute protection.

The question of risk to human health

Scrapie has been known to exist for over 250 years and there is as yet no evidence that there is a human health risk. The concern has prompted numerous intensive studies of the possible epidemiological relationship between scrapie and the human spongiform encephalopathies. The results of many national surveys and other studies do not support a causal link between the diseases in animals and humans.

Matthews and Will (89) reported a case of CJD in a life-long vegetarian. Singhal and Dastur (117) also reported two cases of CJD in life-long vegetarians in India. In looking at the relationship between the occurrence of scrapie and the geographic occurrence of CJD, Masters (88) reported that the incidence of CJD in Australasia, where scrapie is absent, is the same as in Europe and North America. Despite a very high incidence of scrapie in Iceland where consumption of sheep products is high, the incidence of CJD is lower than the world average (116).
An investigation conducted in France over fifteen years concluded that "we neither found an increased risk in people most exposed to sheep or sheep products, nor any relationship between the frequency of CJD and distribution routes of sheep products from scrapie-endemic areas" (8). A well-documented review by Taylor (122) adequately allays any concerns that the causative agents of scrapie and CJD pass readily between the ovine and human species.

Will (132) reported that systematic epidemiological surveys of CJD cases which covered the years 1970 to 1984 in England and Wales showed that the disease occurred in a consistent frequency and distribution pattern. They did not reveal any substantial evidence of case-to-case or zoonotic transmission, nor did they propose any alternative methods of transmission.

BSE has renewed interest and concern about public health and the animal spongiform encephalopathies. In order to monitor the incidence of CJD as well as individual cases, another national surveillance system has been established in the United Kingdom. The monitoring system has been in existence since May 1990. The detailed information obtained between 1970 and 1984 is a useful baseline for the current surveillance. The first year of the survey has shown no significant increase in the incidence of CJD. The European Community (EC) has encouraged similar national monitoring surveys in other countries (132).

**CONTROL**

With limited knowledge of the epidemiology of natural scrapie and the innate characteristics of the disease, control in an endemic country is costly, time-consuming and frustrating. Eradication may be almost impossible. Given the stigma associated with scrapie, the disease is often unreported, making the problem even harder to deal with.

The control and eradication of scrapie is hindered, particularly by the following:

1. Ignorance of the physicochemical nature of the agent. This has prevented the development of a practical, accurate, sensitive, selective and economical method to diagnose the infection in live animals. As a result, the movement of apparently normal sheep may spread the infection during the long incubation period of scrapie.

2. The uncertainties of the role of \( Sip \) gene in the control of scrapie create a risk that the use of selected sires may possibly do more harm than good. For example, a sheep carrying the long incubation alleles of \( Sip \) gene may be infected and it may be actively shedding agent but it may never develop the clinical disease. Such an animal could introduce infection into a flock which would only become manifest as disease in, say, homozygous \( Sip \) sA sheep, several years later (see the section entitled "Genetic influences of agent and host").

3. Lack of knowledge on how to properly rid a natural environment of scrapie contamination.

4. The limited understanding of natural transmission restricts our ability to completely define which animals are exposed and the degree of risk associated with exposure.
These uncertainties and the conflicting theories often cause confusion, frustration and non-cooperation among shepherds.

To achieve success in controlling scrapie, there are a number of important elements which must be included in any programme:

a) a high level of reporting by shepherds and their veterinarians (producer education is important here);

b) reliable records and permanent animal identification;

c) an epidemiological investigation system to perform prompt and thorough investigations and traces;

d) thorough facility clean-up and disinfection;

e) a source of scrapie-free animals for those forced to depopulate;

f) a willingness by the sheep industry to resolve the problem;

g) a marketplace demand for scrapie-free sheep;

h) adequate compensation for producers if excessive losses are incurred as a result of control efforts;

i) ensure that the flock is monitored for a sufficient amount of time.

National efforts to control or eradicate scrapie

Australia and New Zealand

The importation of British sheep caused the introduction of scrapie in New Zealand in 1952 (6) and Australia in 1952 (11). The prompt depopulation by Australia and New Zealand of all the imported sheep, as well as any contacts, led to the eradication of scrapie. They are considered by most countries to be scrapie-free.

Iceland

Scrapie was introduced into northern Iceland in 1878. It was limited thereafter to only this northern region for the first seventy years that it existed. As part of the eradication of other diseases (jaagsiekte, maedi-visna and Johne’s disease) all of the sheep in the scrapie-affected area of Iceland were destroyed during the years 1946 to 1949. Most of the farms which were depopulated were restocked within the same year, but some were left without sheep for up to three years. The disease started to reoccur within two to four years of repopulation. By 1953, not only was part of the original endemic area reinfected but, in addition, scrapie had spread to other areas of the country (116).

Due to the fear that scrapie might spread throughout Iceland leaving no scrapie-free area from which to repopulate, it was decided in 1978 to make another attempt at eradication. This approach called for the total depopulation of all affected flocks, heavily exposed flocks, or groups of flocks in successive quarantine areas. Repopulation was not permitted for at least two years afterwards. During the year prior to restocking, all barns and other facilities, equipment and machinery were thoroughly cleaned by high-pressure soap-washing and then disinfected with a sodium hypochlorite solution. They were then sprayed with an iodophor disinfectant or burned
off with a gas flame. All surfaces in sheep houses and barns up to a height of 1.5 m were sealed with creosote or oil-based paint. All woodwork and even complete sheep houses which could not be disinfected were burned or buried. Surface soil by barns and other heavily exposed areas was removed and replaced with gravel or asphalt. Government inspection was required to ensure that procedures were completed to the standards set by the scheme. In addition, the first harvest of hay from potentially infected fields was not permitted to be used as forage for the new stock. These measures were followed up with comprehensive inspection of flocks, brain examinations, free scrapie diagnosis and, importantly, financial assistance for the disinfection procedures listed above and compensation while owners were out of business (116).

Since 1978, the use, for animal feed, of any offal from abattoirs in scrapie-infected areas has been prohibited.

Extensive surveillance is being maintained on farms and at slaughter (see the section entitled “Epidemiology of scrapie within a flock”) to identify newly-infected flocks quickly. Results of these efforts have been promising. Restocking has been completed in 397 of the total 716 flocks which were destroyed. One hundred and seventy-eight of these have had new animals introduced for at least four years with no evidence of scrapie. There are some farms which have had new sheep for eleven years with no recurrence. Only two of the 397 flocks have been reinfeeted and these were attributed to negligence. Officials of Iceland believe that scrapie can be eradicated there in between 10 and 20 years (116).

United States

The first case of scrapie was diagnosed in 1947 in a Michigan flock. The sheep were of British origin imported from Canada over a period of years.

Efforts to eradicate scrapie in the United States have been in effect since 1952 when the Secretary of Agriculture declared a State of Emergency to deal with the disease. Once the disease was confirmed, the flock was quarantined and then depopulated. All exposed sheep sold from the flock were traced and slaughtered. In 1957 the regulations were amended to include the depopulation of source flocks and all those animals sold from source flocks. Modifications of this approach were made throughout the years; however, the main focus remained on total flock depopulation.

With the adoption of the bloodline/surveillance programme in 1983, emphasis was shifted away from the total flock depopulation approach. The bloodline surveillance programme required that the maternal bloodlines of a scrapie-infected sheep or goat be removed from the flock/herd. The remaining animals were then placed under surveillance for 42 months by government veterinarians for evidence of scrapie. The change was made for a number of reasons:

1. The indemnity costs for total depopulation were high and adequate funding was not always available.

2. It was felt that the drastic measure of total depopulation inhibited owners from reporting the disease and that there was much more scrapie than was reported.

3. A portion of the research community argued against the significance of lateral transmission and stated that most cases of disease spread could be attributed to maternal transmission.
4. After 31 years of a total depopulation policy, scrapie still existed in the United States.

A 1985 United States Animal Health Association resolution prompted a review of the bloodline/surveillance programme. The review panel included representatives from the United States Department of Agriculture/Animal and Plant Health Inspection Service (USDA/APHIS), the research community, State Animal Health Officials and industry. The panel concluded that:

a) the present bloodline/surveillance programme should be abandoned because it was not effective. Lateral transmission within flocks was significant and scientific knowledge was inadequate to effectively eradicate the disease; and

b) the USDA should redirect its efforts and funding towards education and research.

During the 1980s, the number of newly-reported infected flocks per year did increase in the United States. Numerous people commented that scrapie was running rampant in the United States because of the less restrictive bloodline/surveillance programme. Since there was uncertainty about the prevalence of scrapie prior to the change, it was difficult to know whether or not the increase in cases was real. The bloodline/surveillance programme changed a number of factors. Indemnity was increased from a maximum of US$90 to US$300. When scrapie was diagnosed, the producer did not lose the entire genetic base of his flock which may have influenced the willingness to report. During this time, education was stressed throughout the industry making producers aware of the disease and knowledgeable of what to report. On the other hand, there may have been a real increase of infected flocks.

In replacing the bloodline/surveillance programme, the process of Negotiated Rulemaking was used. Due to the fact that there is no test for infection in the asymptomatic animal, regulatory officials would rely heavily on the support of the industry and the cooperation of all involved.

The Negotiated Rulemaking process brought together sheep producers, allied industry representatives, State Veterinarians, scientific advisers and officials of USDA/APHIS to negotiate the new programme. This process was utilised to resolve conflicts between diverse factions and to focus on finding constructive and creative solutions which the industry could support. The Scrapie Negotiated Rulemaking Committee met eight times over nine months to develop the new scrapie control efforts. In January 1991, the Committee passed, by consensus, a core programme for scrapie which included:

1. A one-time depopulation with indemnification for all known infected and source flocks.

2. Regulations to establish types of identification required for the interstate movement of sheep from infected or source flocks.

3. A voluntary flock certification programme — the intent of this programme is to monitor flocks over a period of five years or more and identify flocks which, as far as can be ascertained, are free from scrapie. The programme focuses on risk reduction and sound husbandry practices. It will consist of four levels, with each
advancing level representing a lower risk of scrapie. High-risk animals (designated by criteria of exposure to infection) must be removed if infection is found. The participation requirements are as follows:

Any owner of a flock may apply to enter the **Voluntary Scrapie Flock Certification Program** by sending a written request to the State Scrapie Certification Board.

When participating in the programme, the owner must:

- Agree to report scrapie-suspect animals to the proper animal health official immediately. Such animals must not be sold for breeding or slaughter.

- Officially identify all animals within a flock that are one year of age or older. Animals less than one year old must be identified whenever a change of ownership occurs, except for those in slaughter channels. The proposed identity is an electronic microchip.

- Maintain required records as specified by the programme. Records must be kept a minimum of five years after an animal dies or is removed from the flock.

- Allow breed associations and registries, livestock markets, and packers to disclose records to APHIS and/or State animal health officials.

- After reasonable prior notice, allow inspection of animals and records by APHIS, State animal health officials, and State Scrapie Certification Board members.

- Provide necessary facilities and personnel to assist in inspections, including:
  a) checking animals for official identification and signs of scrapie; and
  b) checking records for completeness and accuracy.

Owners must account for all acquisitions, departures, births and deaths.

- Submit to an official laboratory tissues from scrapie-suspect animals and from animals suspected of other neurological and chronic debilitation illnesses.

The following requirements must also be met:

**Phase 1 – Certifiable Class C**

Minimum time requirement: one year

Flock has annual inspections. Each animal must have a record containing:

- Official identification number and any secondary identification
- Sex
- Breed
- Date of acquisition and source (if animal was not born in flock)
- Disposition – date and cause of death if known; date of removal and destination

**Phase 2 – Certifiable Class B**

Minimum time requirement: two years

The flock has:

- Inspections every six months
- No evidence of scrapie for one year
- Not been found to be a source flock
- Records must also include sire, dam and progeny information

The flock owner has:
- Met requirements of Certifiable Class C
- Agreed to provisions of Certifiable Class B

**Phase 3 - Certifiable Class A**

Minimum time requirement: two years

Inspections, records and identification should be the same as those for Certifiable Class B.

The flock owner has:
- Met requirements of Certifiable Class B
- Agreed to provisions of Certifiable Class A

**Phase 4 - Certified**

Records should be the same as those for Certifiable Class A

The flock has:
- Annual inspections
- No evidence of scrapie for the last five years
- Not been found to be a source flock for the last five years

The flock owner has:
- Met requirements of Certifiable Class A
- Agreed to provisions of Certified status.

All animal acquisitions must be from the same or higher class status, or the flock will return to the status of the lowest status animal acquired. Flock status will be jeopardised if the animals commingle with animals from a flock in a lower phase.

If infection is found in a flock, an epidemiological investigation will be conducted. This investigation will identify trace and source flocks and exposed animals. A flock plan will be developed and implemented.

The flock plan may include depopulation of high-risk animals, reduction of risk associated with spread of infection, and facility clean-up and disinfection.

If one or more animals in a flock is diagnosed with scrapie or identified as a source flock, the flock status will be "pending". After the flock plan is developed and implemented, the flock will return to Certifiable Class C (129).

The new scrapie control programme should enter into effect towards the end of 1992.

*United Kingdom and European Community*

In the past, the United Kingdom and most members of the EC have not had official scrapie control programmes in place; nor was scrapie a notifiable disease in most of these countries. On 1 January 1993, scrapie will become a notifiable disease within the EC.
In addition, countries wishing to trade in breeding stock will be required to have some form of regulated surveillance programme which will stress accurate records and mandatory animal identification. These two elements are essential in tracing the parentage of an animal and also possible sources of infection. Individual flock owners will be compelled to establish freedom from scrapie for at least two years.

The Ministry of Agriculture, Fisheries and Food (MAFF) of Great Britain, in consultation with the Health Scheme Advisory Group, created a protocol for flock owners to comply with the new EC regulations. This control programme is a scrapie monitoring scheme. The standards for this programme are as follows:

1. Owners must complete an application stating that they have read the rules and agree to abide by them.

2. A Veterinary Officer of the State Veterinary Services will conduct a preliminary visit, which may be attended by the private veterinary surgeon (PVS) of the owner. The purpose of the visit will be to assess suitability for membership, and to advise on scheme rules.

3. The State Veterinary Officer conducting the visit will then send a preliminary report to the Divisional Veterinary Officer (DVO) who will then accept or reject the application after considering the recommendation of the Veterinary Officer. The owner will receive this decision in writing. If approved, the date of membership would be the date of the initial visit.

4. An acceptance letter will not only confirm membership, but also establishes the details of work to be carried out during the year. After a flock has attained a year of negative status in the programme, the owner will receive a certificate to document this fact.

Some of the specific rules are outlined below.

**Management:** The owner must maintain a “closed” flock. In terms of scrapie control this means, that to satisfy the requirements of the EC Directive, purchases of breeding female stock must come only from flocks of similar status, i.e. other flocks within the British Sheep and Goat Health Scheme Category IV membership. Males may be purchased from any source although this provision is under review. Members are recommended to seek advice on suitability of such sources.

Flock security must be such that no straying can occur into or out of the premises.

Shared or common grazing is not permitted unless all sharers/commoners agree to participate in the Scheme, in which case a reactor in any one flock would destroy the status of all the other flocks.

**Marking:** All animals would need to be individually and permanently marked. To prevent loss of identification, this involves double tagging or tagging and tattooing.

**Records:** Records must be kept of:

a) a full flock list of individual animals;

b) dam and sire of all animals born;

c) all movements on and off, identified by individual markings;

d) all deaths of animals over 12 months of age;
e) all live births (within 7 days of birth);
f) agistments;
g) hirings, e.g. rams.

These records must be made available at the annual flock inspection and at any other reasonable time that the PVS or Veterinary Officer may require. They must be kept for a period of ten years after the year of membership in which they are made. These records are in addition to any records required to be kept under existing statutory legislation.

**Flock inspection:** At least once every twelve months, the PVS shall carry out a flock inspection. This will involve gathering the flock and inspecting and recording all individual identifications, together with a clinical inspection of all animals for clinical signs of scrapie. At the flock inspection visit, flock records will also be inspected and audited, together with the individual flock inspection records to ensure that all losses are accounted for.

**Cull inspections:** All animals over the age of two years which are being culled, must be inspected by the PVS before leaving the premises. This includes animals being sold as potential draft breeding stock.

A representative sample of cull stock, selected by the PVS out of the culls, must be sent to slaughter to ensure that histopathological examination of brain material is carried out. A minimum requirement for this is one animal per flock per annum for flock sizes up to 100 breeding sheep and one per 100 thereafter.

In small units where culling is not carried out every year, all cull animals sent for slaughter shall be made available both for clinical inspection and histopathological examination, the number submitted for the latter to be at the discretion of the PVS.

**Suspect cases:** All animals showing evidence of nervous disorder or pruritus must be reported to the PVS who shall inspect the animal and, if necessary, based on clinical assessment, arrange for the animal or carcass to be sent to the VI Laboratory where histopathological examination of the brain and disposal of the carcass shall be carried out free of charge. The PVS shall be responsible for informing the DVO of all suspect cases examined and the outcome of the examination and histopathological examination where appropriate.

**Histopathological reports:** The owner shall be under an obligation to ensure that, as part of his culling programme, he makes material available to the PVS to forward to an approved laboratory, such that histopathological examination of brain material from the cull(s) selected by the PVS at the cull inspection is carried out to confirm absence of scrapie-induced spongiform encephalopathy.

**Control of scrapie within a flock**

Current scientific information considers placenta (108, 109) and possibly associated fluids as primary sources of agent. Hence, control measures within a flock should revolve around sound husbandry practices especially at the time of lambing. Placentas should be removed promptly and any animal showing signs which are even slightly suggestive of scrapie should not be allowed to lamb with the rest of the flock.
It was frequently observed in the United States that the most likely animals to develop scrapie were offspring of an infected ewe. Other sheep born during a season in which an infected ewe lambed were also at high risk of becoming infected, particularly those born around the same time period and in close proximity to the infected parturient ewe (Detwiler, unpublished observations). It is suggested that these "high-risk" animals be identified and removed from the flock as soon as possible. Such action requires precise record-keeping over many years. The lambing premises and equipment should be thoroughly cleaned of organic matter and it is then recommended that they be exposed to 2% available chlorine (19) or a 1 molar sodium hydroxide (121) at ambient temperature for at least 30 min. These recommendations are based on work in the laboratory and it is not certain that they would be 100% effective in the field. More research needs to be conducted in this area.

When more is learned about the roles of the Sip and PrP genes in controlling scrapie, sire selection may play a significant part in the control of scrapie within a flock.

CONCLUSION

Scrapie is a very complex disease (both scientifically and politically) and often confusing to deal with. From a scientific standpoint, the causative agent has yet to be defined, the route of natural transmission is not fully understood, there is no guaranteed prevention, there is no treatment, and there is no practical and effective test for the non-clinical or even clinically-ill live animal. Regulatory officials are charged with taking all of these unknowns, some of which are the object of conflicting theories, and making sound, effective policy with limited funding. This can be a monumental undertaking.

Most of the current recommendations for the control of scrapie are based on actual knowledge as well as speculation. It is important that government control programmes are sound and use what is known to reduce as many risks of disease transmission as possible. Further research is needed to:

a) develop a preclinical live animal test;

b) determine the occurrence of infected carriers in relation to different genotypes of sheep and what role (if any) they play in the dissemination of scrapie;

c) determine the potential use of rams of a certain genotype to control the natural disease;

d) determine what role the environment plays in the spread of scrapie and if there is a significant risk provided by the environment, how one negates that risk.

As new information becomes available, control and eradication programmes need to be adjusted accordingly. Above all, it is essential that flock owners, regulatory officials and private veterinary practitioners work in cooperation with each other if scrapie is to be effectively controlled or eradicated.

** **
LA TREMBLANTE. — L.A. Detwiler.

Résumé: L'auteur rappelle l'historique, la répartition géographique, l'origine, l'épidémiologie, les symptômes cliniques, la pathogénie, les lésions, le diagnostic, la prévention, la prophylaxie et les implications économiques de la tremblante chez le mouton. Une brève description de la maladie chez la chèvre et le mouflon et la nature de l'agent causal, le contrôle génétique de la durée d'incubation de la maladie chez le mouton ainsi que les modalités de la transmission naturelle de la tremblante chez le mouton et la chèvre sont discutés. Les programmes nationaux de prophylaxie de la maladie dans divers pays sont indiqués.


PRURIGO LUMBAR. — L.A. Detwiler.

Resumen: El autor presenta un informe detallado de la historia, distribución geográfica, causas, epidemiología, sintomatología, patogenia, patología, diagnóstico, prevención, control y efectos económicos del prurigo lumbar de los ovinos. Se menciona brevemente la enfermedad en los caprinos y en los musmones. También se discute la naturaleza del agente etiológico del prurigo lumbar, el control genético del periodo de incubación en los ovinos y la transmisión natural del prurigo lumbar en los ovinos y los caprinos. Los esfuerzos nacionales por controlar el prurigo lumbar en varios países también están indicados.

PALABRAS CLAVE: Análisis - Control de la enfermedad - Encefalopatía espongiforme - Enfermedad causada por un prion - Enfermedades de los ovinos - Frecuencia de la enfermedad - Prurigo lumbar.

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