Risks of transmitting scrapie and bovine spongiform encephalopathy by semen and embryos

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Summary

This paper reviews current knowledge on transmission of scrapie and bovine spongiform encephalopathy (BSE) by semen and embryos. In sheep, in particular, it is difficult to distinguish between the genetic transmission of susceptibility to scrapie and vertical transmission of the infection. Nevertheless, there is evidence that vertical transmission of infection does occur, probably across the placenta, but none to suggest a significant scrapie risk from semen.

Two teams have studied scrapie transmission from experimentally infected sheep using embryo transfer. Whereas one team found no evidence for transmission, the results from the other team suggest that embryos, even after washing, might carry the disease into the offspring.

In regard to goats, although genetic differences in susceptibility exist, they are much less obvious than in sheep. There is no evidence for vertical transmission or for transmission through semen and embryos.

With regard to BSE, although it appears that genetic differences in susceptibility are absent or unimportant, some recent work does suggest that the disease may be passed from cow to calf. The route of transmission and stage or stages when this takes place are unclear, however.

In conclusion, despite growing evidence to indicate that scrapie and BSE are unlikely to be transmitted through semen and embryos, more research is needed to confirm this. Furthermore, until all possibility of risk is ruled out, risk reduction methods must be considered, especially when semen and embryos are being imported into countries where the diseases do not exist.

Keywords


Introduction

Recent events concerning bovine spongiform encephalopathy (BSE) and the human equivalent, Creutzfeldt-Jacob disease (CJD), have focused world-wide attention on these and other transmissible spongiform encephalopathies (TSEs), including scrapie. Scrapie, the disease of sheep and goats, is regarded as the prototype for the TSE group of diseases. The enigmatic nature of their causal agents, and the extremely long incubation periods prior to onset of clinical manifestations (which are invariably fatal), put TSEs into a unique category. Veterinary authorities are especially conscious of the risks of importing these diseases. The absence of practical tests to detect infection in live animals means that cases may appear years after importation, and long after the end of quarantine periods used to prevent the introduction of conventional infectious diseases.

Historically, scrapie has been introduced into several countries by the importation of live, apparently healthy sheep (17). In the 1950s, in two unrelated incidents, the disease...
occurred in Australia and New Zealand among batches of imported animals. Following diagnosis, the imports together with all in-contact sheep were immediately slaughtered, and since then, apart from one outbreak in quarantine in New Zealand in the 1970s, there have been no further cases in those particular countries (74, 85). Some other countries, however, have been less fortunate, in that, following the occurrence of scrapie among imported sheep, the disease became endemic. The enormous difficulties of attempting to eradicate scrapie once it becomes endemic are illustrated by experiences in Iceland (100), where the disease was introduced through sheep importations over a century ago. The inability to detect cattle which were presumably incubating BSE at the time of their importation from the United Kingdom (UK) has also led to isolated cases of this disease in several countries, including Canada, Denmark, Germany, Italy and Oman (81). In contrast to scrapie, however, all evidence so far indicates that the risk of BSE becoming endemic as a consequence of such importations is minimal if feeding and recycling of infected meat-and-bone meal are avoided. Imported cases are nevertheless troublesome and expensive to deal with.

The purpose of this paper is to review what is known about certain aspects of scrapie and BSE and to ascertain whether the acknowledged transmission risks associated with moving animals might be overcome by moving embryos and semen instead. If credible assurances could be given that, with appropriate precautions, the risks of transmission by artificial insemination (AI) and embryo transfer were negligible, major trading advantages would accrue. Benefits would also arise from the use of AI and embryo transfer in domestic control programmes for these diseases.

Current research and changing perceptions

Prior to the recognition of BSE in 1986 (112), there was already a substantial literature on sheep scrapie and on experimental models in laboratory animals. Fortunately, many excellent reviews are available, including those in the special edition of the Office International des Epizooties (OIE) Scientific and Technical Review devoted to TSEs (7). To avoid undue repetition here, some aspects of TSEs are omitted or covered only briefly to give background information and to update with more recent observations. The BSE epidemic in the UK has prompted a huge increase in research, so there is much new information to cover. Relevant publications are appearing so fast that reviews such as this tend to be out-of-date before they are published.

A mere five years ago, it seemed reasonable to conclude (123) from the work of Foote and his colleagues in the United States of America (USA) that the risks of transmitting scrapie by semen and embryos were negligible. BSE was still a very new disease, and information about it was largely speculative, surmised by analogies with scrapie and other TSEs. The accepted view, however, was that cases of BSE probably arose solely from consumption of contaminated meat-and-bone meal (116). Available evidence indicated that transmission of BSE from mother to offspring occurred rarely, if ever, and was of little consequence, especially so far as maintenance of the epidemic was concerned (55). It was logical to assume, therefore, that the risk of BSE transmission by AI and/or embryo transfer would also be negligible. It seemed that for both scrapie and BSE, as for most other infectious diseases, if appropriate sanitary precautions were taken, semen and embryos could be moved virtually without risks between affected and unaffected farms, and from affected to unaffected countries.

Perceptions of these risks have now changed dramatically. Results of embryo transfer experiments in Scotland, published in 1992, 1994 and 1996 by Foster et al. (33, 35, 37), appear to show that scrapie can be transmitted via sheep embryos. Moreover, in August 1996, interim results of a large cohort study on maternal transmission of BSE were issued (1) and interpreted as suggesting that transmission by this route may occur to a significant extent in cattle. It is timely, therefore, in view of these new findings, to reassess the possibilities of transmission of scrapie and BSE by semen and embryos.

The infective agents of scrapie and bovine spongiform encephalopathy

Despite decades of research to characterise them, the precise molecular nature of the agents causing TSE infections remains uncertain. Like viruses, they can be transmitted from host to host, but they do not seem to resemble viruses biochemically, being extremely resistant to heat, irradiation and most of the disinfectants known to inactivate conventional viruses.

Whatever the agents may be, when they reach the central nervous system (CNS), the effect is to change the natural, soluble, cell membrane protein PrP\textsuperscript{Sc} into an abnormal, insoluble and protease-resistant form, PrP\textsuperscript{Sc}. One theory which has gained wide acceptance recently is that the molecules of PrP\textsuperscript{Sc} (referred to as 'prions') are themselves the agent, and that these 'seed' the further conversion of PrP to PrP\textsuperscript{Sc} (94, 95). Exponential formation of PrP\textsuperscript{Sc} gives the appearance of a very long incubation period followed by a terminal, clinical phase which is ultimately fatal. Accumulation of abnormal PrP\textsuperscript{Sc} in neurones leads to brain degeneration with the characteristic spongiform changes which are seen histologically in all TSEs.

A difficulty with the 'prion (protein only)' hypothesis is that different strains of TSE agent usually maintain their stable biological characteristics (i.e. the disease characteristics induced in the primary host), even when passed through other host species. This is hard to reconcile with the view that
the agent is devoid of a replicating, information-carrying (i.e. genetic) molecule, such as nucleic acid, and led Dickinson and Outram (25) to put forward an alternative hypothesis, that the molecule in question does exist but is extremely small and is protected by the host PrPSc protein (the ‘virino’ hypothesis). Although many scientists now favour the ‘prion’ hypothesis and believe that the informational molecules could consist solely of protein (118), not all are convinced that nucleic acid molecules are absent (9, 103).

**Strains and strain typing**

Irrespective of their biochemical nature, it is important to note that many strains of TSE agent exist. Thus, there are approximately 20 phenotypically distinct strains of scrapie and another distinct strain of BSE. Scrapie strains fall into two groups, A and C, and this classification depends on their interaction with different genotypes of mice and sheep. The so-called SSBP-1 ('sheep scrapie brain pool') inoculum, originally used in 1950 (119) and still used in research, including in the sheep embryo transfer experiments described later, is thought to contain a mixture of at least three A strains (18). Currently only one scrapie strain, CH1641, is known to behave as a group C strain but, when the BSE agent is inoculated into sheep, this also behaves like a group C strain.

Although not all scrapie strains are transmissible to mice, most are, and current laboratory methods for identifying or typing strains involve measurement of disease characteristics (e.g. incubation periods and the severity and distribution of brain lesions) following injection of samples into mice of various defined genotypes (9). Bioassays, as these are called, can be used to detect whether infectivity is present in a sample. It is also possible to titrate levels of infectivity by injecting mice of standard genotype with a range of dilutions and then measuring the incubation periods. Incubations in mice vary from about 150 days to the full extent of their life span of over 1,000 days, but this depends on agent strain, dose and the mouse genotype. If a standard dose of a specific TSE strain is given intracerebrally to groups of genetically uniform mice, their incubation periods tend to be very uniform and predictable.

Control of incubation in mice is mediated mainly by the Sinc gene, which encodes PrP and has two alleles: Sinc<sup>s7</sup> and Sinc<sup>p7</sup>. With most mouse-adapted scrapie strains, the incubation period is short in Sinc<sup>s7</sup>s<sup>s7</sup> homozygotes, long in Sinc<sup>p7</sup>p<sup>p7</sup> homozygotes and intermediate in heterozygotes, but in a few strains the situation is reversed. Once the agent strain has been characterised, it is usual to use mouse strains of known susceptibility for detection and diagnosis, as well as for experimental purposes. For example, in BSE experiments the homozygous Sinc<sup>s7</sup> RIII and Sinc<sup>p7</sup>C57BL strains are often used. In addition to mice, various other species are susceptible to TSEs and some, such as hamsters and monkeys, may be used in experimental studies. Hamsters, unlike mice, do not show genetic differences in incubation periods and are unsuitable for strain typing, nevertheless they are useful because they often have very short incubations and produce high titres of infectivity in brain tissue (3).

Although bioassay is the only available method for demonstrating and quantifying TSE infectivity, it should be noted that when positive samples from one species are injected into another species, the incubation period is usually longer and the sensitivity lower than for within-species transmissions. This phenomenon, known as the 'species barrier effect' (18), is important and must be borne in mind when samples from, say, sheep, goats or cattle are bioassayed in mice for scrapie or BSE infectivity. Statements to the effect that specified tissues from affected animals 'contain no infective agent' when bioassayed in mice must be treated with caution because very low infectivity levels may not be detected in mice. The obvious alternative is to use cattle, goats or sheep for the bioassays, but this is both expensive and slow, and reliable sources of infection-free animals may be difficult to find.

**Host genetic factors in sheep, goats and cattle**

Inherited differences in susceptibility to TSEs, particularly scrapie in sheep, have long been known about, but the last few years have seen major advances in understanding the genetic mechanisms involved. These are of special relevance to the possibilities of TSE transmission via semen and embryos.

**Sheep**

Early experimental studies on scrapie in sheep gave some surprising results. One such study was the so-called 'twenty-four breed' experiment of Gordon in the 1950s, in which groups of about 40 sheep of each of 24 British breeds were injected with the SSBP-1 scrapie inoculum (47). Subsequently, over a two-year observation period, the proportions developing scrapie varied from 74% in the Herdwick breed to zero in the Dorset Down breed. Later work revealed that susceptibility was not simply a breed factor but also varied within families of the same breed. For example, some of the Dorset Downs went on to develop scrapie when they were over two years old, while in some Herdwick flocks high proportions of resistant sheep were found (18). These early studies led to the creation in Britain of research flocks consisting of three main breeds for genetic selection studies: Cheviots, Herdwicks and Swaledales. Work with these and other breeds around the world has shown that scrapie susceptibility is a very complex topic.

It became apparent, for example, that incubation in sheep is largely controlled by a Sip ('scrapie incubation period') gene, with two alleles, sA and pA, which respectively shorten and
both mice and sheep have shown that, following infection, the incubation periods in scrapie A or C strains are shown in Table I. Studies in both mice and sheep have shown that, following infection, the agent develops and persists in peripheral (extraneural) tissues, but clinical disease only occurs when the agent spreads to the CNS. In mice the Sip gene appears to control multiplications of scrapie in the CNS but not in peripheral tissues, and it is possible that the Sip gene may act similarly in sheep. If so, it follows that scrapie A strains may not always spread to the CNS in Sip pApA homozygous sheep, and this might be why no clinical signs are seen. Such animals may, nevertheless, become carriers of infectivity in their peripheral tissues and might be a source of environmental (and perhaps transplacental) contamination.

Table I: Incubation periods in Cheviot sheep of different Sip genotypes when given scrapie inocula SSBP-1 (group A strains) or CH1641 (group C strain) (21, 32)

<table>
<thead>
<tr>
<th>Scrape source</th>
<th>Route of injection</th>
<th>Incubation period (days ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSBP-1</td>
<td>Intracerebral</td>
<td>Positive line: 197 ± 7 (Sip sAsA or sApA)</td>
</tr>
<tr>
<td>SSBP-1</td>
<td>Subcutaneous</td>
<td>313 ± 9</td>
</tr>
<tr>
<td>CH1641</td>
<td>Intracerebral</td>
<td>595 ± 122</td>
</tr>
</tbody>
</table>

SEM: standard error of the mean

Sip genotyping was originally based on the fate in phenotypic terms (i.e., long or short scrapie incubation periods) of individuals in families of sheep under study. Now, although Sip genotypes are sometimes referred to, it is known that incubation and other scrapie characteristics in sheep are controlled not in simple Mendelian fashion by a single polymorphism, but by at least two or three distinct polymorphisms located on separate codons within the PrP gene. This gene, which is present in all mammals, codes for PrP protein, and there is good evidence that the Sip and Sinc genes, in sheep and mice respectively, are in fact the same as the PrP gene. The Sip concept therefore, originally dependent on cumbersome, long-term phenotypic observations, is now being superseded by the much more precise techniques of fingerprinting deoxyribonucleic acid (DNA) sequences on the PrP gene. The gene is extracted from blood or other tissue samples and then amplified by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis to read the DNA coding sequence. Examination of the sheep PrP gene coding sequence has revealed that the main polymorphisms are located at codons 136, 154 and 171, with alanine/valine (Ala/Val), arginine/histidine (Arg/His) and glutamine/arginine (Gln/Arg), respectively, being encoded at these loci (13, 60). The most important codons seem to be those at 136 and 171.

In Cheviot sheep in the Edinburgh Neuropathogenesis Unit (NPU) flock, and in at least some Cheviots in the USA, codon 136 of the PrP gene is the most strongly linked with phenotypic manifestations of Sip. Thus, Sip sAsA genotype Cheviots encode valine at 136 and are referred to as Val/Val-136, whereas Sip pApA Cheviots encode alanine and are Ala/Ala-136 (44, 45, 60, 72, 75). Cheviots with Val/Val-136 genotype, therefore, are highly susceptible to experimental challenge with group A scrapie strains, and the heterozygotes with Val/Ala-136 are partially susceptible.

Natural scrapie also tends to occur predominantly in Val/Val-136 genotype sheep in the Swaledale, Shetland, Ile-de-France and some other breeds (4, 13). However, not all scrapie-affected breeds encode valine at PrP codon 136. In Suffolks and some other breeds like the Romanov and Lacaune, for example, virtually all are Ala/Ala-136, and polymorphism at codon 171 is by far the most important. In this case, it is Gln/Gln-171 individuals which are highly susceptible to the disease, whereas Arg/Arg-171 sheep are resistant. To complicate matters further, codon 154 is associated with scrapie incidence in NPU Cheviots (63). Thus, whereas some Val/Ala-136; Gln/Gln-171 Cheviot (NPU) sheep will succumb to natural scrapie, this depends on their genotype at 154. His/His-154 and His/Arg-154 animals survive, but those with Arg/Arg-154 tend to develop the disease. It is clear from these recent genotyping studies that it is important in sheep not only to know the breed, but also the full PrP genotype, before gauging the likely susceptibility to scrapie A strains.

**Goats**

Early work with large numbers of goats infected mainly through the intracerebral route with SSBP-1 inoculum (47, 88, 90) revealed a high (near 100%) susceptibility. Consequently, goats came to be regarded as virtually always susceptible to scrapie. Probably owing to the species barrier, however, the incubation periods were variable in those goats (15 to 22 months), and after further passages they fell to an average of about 12 months, with some as low as eight months. Transmission was also achieved by oral dosing (89). Recent studies on the caprine PrP gene (46) have revealed the existence of polymorphisms at codons 142, 143 and 240, with codon 142 having quite a marked influence on incubation periods in goats infected experimentally with certain scrapie strains, and with BSE.

**Cattle**

Biometrical work involving large numbers of cattle in the British epidemic has so far produced no significant evidence of breed, pedigree or sire effects on the incidence of BSE (16, 68, 113). The constancy of histological lesions in brains of affected cattle from a wide variety of British breeds (101) also
seems to point not only to the existence of a single, stable strain of the agent, but to an absence of host genetic factors as well. At least two polymorphisms have been found in the coding region of the bovine PrP gene (62,77,82), which indicates that different genotypes and variant forms of PrP protein exist, although none of them seems to predispose cattle to BSE infection. Thus, in studies with fairly large numbers of cattle, no differences were found between the PrP genotypes of the healthy cattle and those with the disease (62). It is conceivable that a link between some other as yet unknown PrP polymorphism and BSE susceptibility may exist (62), but nothing equivalent to the Snc or Sp gene is currently known.

Transmission between animals

A matter that has perplexed scrapie researchers for many years, and BSE researchers in the last decade, is how these diseases are transmitted. Of course, in this paper the main focus is on the possibility of transmission via semen and embryos, but to avoid semantic confusion, references to transmission routes are essentially as cited by Detwiler (17), as follows:

- lateral or horizontal transmission: the spread of infection between unrelated animals via direct or indirect contact at any time, or to offspring after parturition

- vertical transmission: the spread of infection, or genes responsible for disease, from parent to offspring via germ plasm (spermatozoa or ova) at the time of fertilisation or in utero during embryonic and foetal development

- maternal transmission: the spread of infection from a dam to her offspring either vertically (e.g. via female germ plasm, or across the placenta), or horizontally in the immediate post-parturient period (via milk, saliva, faeces, etc.).

Whereas it is quite easy to transmit scrapie and BSE experimentally to susceptible animals by injecting them with infected brain material, this is of doubtful relevance to understanding natural routes of infection. If natural transmission is to occur, replication of the agent must take place in peripheral (extraneural) tissues and eventually be excreted or otherwise passed out of the body. To understand transmission via semen, embryos or indeed any other route, therefore, it is necessary to know what tissues are involved in replication or carriage of these agents. Potential candidates for this role are numerous and their identification is usually conducted by injecting them into mice. It should be emphasised, however, that merely testing for infectivity by mouse inoculation is not enough to distinguish between tissues containing traces of the agent and those that could be major sources or routes for shedding to the exterior; this requires titration.

Particularly relevant to the risks of transmission by semen and embryos, and also to preventing such risks, is whether at any stage in the long incubation, and in the clinical period, the infection might be present in reproductive tissues and gametes. Also important is the possibility of infectivity in blood, body secretions and excretions. Thus, a knowledge of the pathogenesis of these diseases is fundamental.

Pathogenesis

Much original research into the pathogenesis of scrapie and other TSEs was performed in the 1960s and 1970s using laboratory animals, especially mice and hamsters. Research was also conducted on sheep and goats, but again using mainly experimentally induced infections. It was not until the 1980s that detailed studies commenced on the pathogenesis of natural sheep scrapie, and these were almost solely on the Suffolk breed (see below). Consequently, there are still major gaps in the knowledge of scrapie pathogenesis in sheep. Work on the pathogenesis of BSE is progressing but, for obvious reasons, is still at an early stage.

Laboratory animal models

The discovery in the early 1960s (11) that scrapie can be transmitted to mice was an important breakthrough. However, in contrast to sheep, natural transmission between mice is extremely rare, the only reported cases apparently being due to wound contamination caused by fighting (47). For experimental purposes, including bioassays, mice are often exposed to infection by intracerebral injection, but several other routes have been used, including the oral, subcutaneous, intraperitoneal, intravenous and conjunctival routes and skin scarification. After exposure by any of these non-neural routes, the agent replicates in the lymphoreticular tissue of Peyer’s patches in the small and large intestine, in mesenteric and other lymph nodes, and in the spleen (70,109). Sequential studies in mice (27) revealed that, after subcutaneous injection of scrapie, the infectivity seemed to disappear for a time and was undetectable by bioassay. This phenomenon, which also occurs in other species, became known as the ‘eclipse phase’ (24). Subsequently, after about four weeks, infectivity was detected at increasing levels in the spleen and lymph nodes. Similar rises occurred in the thymus and submaxillary salivary glands from eight weeks, and at 16 weeks the scrapie agent began to accumulate in the brain. Titres in peripheral tissues then reached a plateau, but those in the brain and spinal cord continued to rise, and by the onset of the clinical stage they were more than ten times those found in peripheral tissues.

There is evidence in mice that scrapie infection may travel from the abdominal lymphoreticular tissues along peripheral (sympathetic) nerves to infect the mid-thoracic spinal cord, from where the infection eventually spreads to the rest of the CNS (69). Progression along the optic nerve has also been reported in mice following intraocular exposure (42). Another possibility, for which there is evidence but over which there is also some controversy, is that infection might be carried via the bloodstream. If haematogenous carriage is involved, this would have important implications because virtually all
tissues, including semen, embryos and tissues of the reproductive tract, would be potentially infectious.

Early mouse studies involving fractionation of spleen cells indicated a possible association between scrapie infectivity and lymphoblasts, myeloblasts and macrophages. It has since become clear, however, that the lymphoreticular cells involved in scrapie replication are more likely to be long-lived cells, which are independent of stem cell replacements (38, 39). One candidate is the follicular dendritic cell (FDC), thought to be involved with trapping antigenic material and stimulation of B-lymphocyte production (8, 73). Recent investigations into scrapie pathogenesis have involved a mutant strain of mice, known as the severely combined immunodeficient (SCID) strain, in which the FDCs are non-functional and B- and T-lymphocytes are absent (41). SCID mice are relatively resistant to scrapie infection (mouse passaged strains) by peripheral challenge, and replication cannot be detected in their spleens, but if challenged intracerebrally they do develop the disease, as do normal mice.

Another recent suggestion is that platelets might have a role in replication and carriage of TSE agents (93). Platelets share some biochemical characteristics with neurons (e.g. both express PrP\(^\text{Sc}\) on their surface) and thus could conceivably constitute a matrix for the conversion of PrP\(^\text{Sc}\) into the pathological form, PrP\(^\text{Sc}\), in which case such platelets might act as haematogenous carriers of the agent. The spleen, which has a significant role in pre-neural replication of the scrapie agent, is a major site of platelet storage (64).

### The question of haematogenous spread

As mentioned above, the question of whether TSE infectivity spreads by way of the blood stream is of great significance. Low, transitory levels of infection have been reported in whole blood and serum of mice shortly after their exposure to scrapie (28, 80), and in mice and rats at the onset of clinical disease (12, 22, 28). However, because tissue damage is almost inevitable when collecting blood, it is difficult to rule out the alternative explanation that the infectivity arose from contamination with fragments of other infected tissues.

More convincing evidence of scrapie infectivity in blood has come from work with hamsters. Compared with those in other species, incubation periods in hamsters tend to be extremely short and can be as little as 60 days, which is less than half the fastest period in mice (71). Another feature of hamster scrapie is that sustained 'viraemias' occur. In a study of hamsters which had been injected with scrapie intraperitoneally, Casaccia et al. (10) detected infectivity in samples of concentrated blood for up to 90 days post-injection, but not at 100 days when clinical disease commenced. Two points about this work are noteworthy; the first is that the blood was highly concentrated prior to testing, and the second is that the infectivity was detected by bioassay in other hamsters, thus avoiding the insensitivity that could have arisen from the use of a non-homologous species.

Whether haematogenous spread may be involved in pathogenesis of TSEs in sheep, goats and cattle is still unknown, but most research so far indicates that it is unlikely. Apart from an early report of transmission of scrapie-like illness to mice by inoculation of serum from an affected ram (43), scrapie appears not to have been detected in blood or serum from sheep and goats, or in blood clots, serum or the Buffy coat from cattle affected with BSE. It is important to reiterate, however, that failure to detect infectivity does not necessarily mean that it is absent. Tests on blood concentrates and on specific blood cells from preclinically and clinically affected animals of these livestock species have not been numerous, and current methods may be incapable of detecting very low and intermittent levels of infectivity. Owing to the species barrier, this would be especially true where the blood is bioassayed in mice, rather than in the natural host species.

### Tissue infectivity studies in sheep

Pathogenesis studies in sheep have yielded results broadly in accord with those in mice, although there are some differences. Probably the most detailed work was by Hadlow et al., who examined natural scrapie cases from high-risk families of Suffolks in the experimental flock at Mission Experimental Station, Texas, USA (51, 53). Infectivity titres were measured by intracerebral injection of susceptible, random-bred Swiss mice. Initially, these researchers looked at tissues from young sheep prior to the onset of clinical signs, including lambs at birth, at three months old, at seven to eight months, at ten to 14 months and at 25 months old. Infectivity was first detected in the ten-to-14-month-old animals (eight of 15 were positive), where it was consistently present in the retropharyngeal and mesenteric lymph nodes and the spleen, and occasionally present in the tonsil, prescapular and prefemoral lymph nodes, ileum and proximal colon. One of three 25-month-old sheep, although still clinically normal, was shown to be infected, with the scrapie agent widespread in the lymphatic tissues (including tonsil), spleen, ileum and proximal colon, and with low titres (but no histopathological lesions) in parts of the brain. Hadlow believed the primary exposure to scrapie in these cases was via the oral route, with initial replication of infection in the oropharynx, retropharyngeal lymph nodes, intestine and mesenteric lymph nodes.

In their next study, Hadlow et al. (53) examined nine clinically ill, natural scrapie cases in Suffolks aged from 34 to 57 months. Characteristic brain lesions were present post mortem in all these animals. Infectivity titres in the brain and spinal cord were consistently high or very high, and moderate levels were always present in the spleen, tonsil and lymph nodes (retropharyngeal, bronchial mediastinal, mesenteric-portal, prescapular and prefemoral), and in the ileum and proximal colon. Smaller amounts were often detected in cerebrospinal fluid, the pituitary gland, distal colon, adrenal gland and sciatic nerve and, in a few cases (three or fewer of the nine sheep), traces of infectivity were...
also detected in the nasal mucosa, pancreas, liver, bone marrow, supramammary lymph node and thymus. Mouse bioassays on a range of other tissues, including blood clot, salivary gland, saliva, heart, lung, kidney, thyroid, skeletal muscle, testes, seminal vesicle, mammary gland, ovary, uterus, placenta and foetuses gave negative results.

The inability of Hadlow et al. (53) to detect infectivity in the placenta and foetuses is worthy of special comment. Four of their affected ewes were pregnant, and tissues, including brain, thymus, liver and spleen from the larger foetuses, and pooled brain and abdominal viscera from smaller ones, were examined by mouse inoculation, as were placental cotyledons from two ewes; all with negative results. These are in contrast with the better known, earlier findings of Pattison et al. (91, 92), who demonstrated placental infectivity by inoculation of susceptible sheep and goats rather than mice. The placental tissues used in this study were from six Swaledale ewes in late pregnancy with confirmed scrapie, and samples of these were administered to sheep (Herdwick breed) and goats either by intracerebral injection or oral dosing. In the subsequent observation periods (58 months for sheep and 30 months for goats), 10 of 12 injected plus 9 of 11 dosed sheep, and one of 18 injected plus 3 of 10 dosed goats developed scrapie. On this evidence, all six placentas were denoted positive by Pattison (92). If this were so, it is surprising that the proportion of goats affected was not higher. Moreover, the facts that the incubation periods in the sheep varied greatly (21 to 57 months) after their placental exposure, and that no difference was seen between the incubation periods of the injected and the dosed sheep, suggest that some of the animals may have picked up natural scrapie infection from the environment.

With hindsight, and particularly with the new knowledge on genetic control of incubation, the variable interval to scrapie onset in these sheep is not especially surprising. Nevertheless, scepticism about the placental infectivity work of Pattison has been expressed. For example, Ridley and Baker (98) point out that, in another paper (87), Pattison mentioned that the flock of Herdwick sheep used for the placental infectivity bioassays had been bred for high susceptibility. Thus, since natural scrapie cases obviously occurred in that flock, some or all of those inoculated with placenta were probably destined to develop scrapie anyway. Obviously, doubts as to the validity of the results obtained by Pattison on scrapie infection in placenta must be recognised, but the fact that one of the goats which was injected intracerebrally with placenta and three of those which were dosed orally developed the disease does give the work some credibility.

In addition to their studies on Suffolk sheep, Hadlow et al. conducted some tissue infectivity studies on certain other breeds, notably the Cheviot and related Montadale (51), but the results were not so consistent. For example, in two clinically affected Cheviot ewes the agent was less widely distributed and the infectivity levels were much lower than in the Suffolks. Moreover, in one Montadale ewe, despite histopathological confirmation of scrapie and the presence of moderate levels of infectivity in the brain, there was a complete lack of detectable infectivity in the peripheral tissues. It is difficult to conclude very much from these limited findings on breed differences. They may be a consequence of host genetic factors, but the fact that such differences exist has important implications for risk management, as will be seen later.

Mouse bioassays have been used by some other workers to detect scrapie infectivity in ovine tissues. Hourrigan, for example (56, 57), lists tissues from experimentally and naturally affected sheep of various ages which were tested during several years of work at Mission Station, Texas. In addition to CNS, his list of positive tissues includes the sciatic and dental nerves, salivary gland, pituitary, adrenal gland, muscle, bone marrow, thymus, spleen, various lymph nodes, intestine (ileum and proximal and distal colon), kidney, nasal mucosa and lung. Infectivity in several peripheral nerves (Nervus ischiadicus, N. medialis, N. tibialis, N. fibularis, N. axillaris and N. ulnaris) has also been reported in a recent study (49). Apropos the reproductive tissues, Hourrigan records that low levels of infectivity were found in the ovary (4 of 14 tested), uterus (4 of 13), uterine caruncle (1 of 10), amniotic fluid (1 of 1), foetal cotyledon (2 of 10), and foetus (1 of 13), but none was found in 6 testes or in 21 samples of semen. Absence of technical detail in these reports (56, 57) makes it difficult to gauge their significance, and consequently there is some scepticism about these findings.

### Tissue infectivity studies in goats

Contemporary with their pathogenesis studies in sheep, Hadlow and colleagues reported work of a similar nature, and with essentially similar results, in three naturally affected dairy goats with clinical scrapie (52). Infectivity titres were high in the CNS, moderate in the pituitary, tonsil, lymph nodes, spleen, ileum and proximal colon, and low in the adrenal gland, thymus, distal colon and nasal mucosa from all three goats. Traces of infectivity were detected in the lung of one goat but the serum, blood clot, milk, faeces, skeletal muscle, bone marrow, kidney, salivary glands, ovary, uterus (caruncle) and mammary gland were all negative. In an earlier study, Pattison and Millson (90) had detected infection in the salivary glands of clinically affected goats but not in their saliva.

### Infectivity studies on tissues from cattle with bovine spongiform encephalopathy

A wide range of tissues from natural BSE cases has been tested for infectivity using sensitive mouse bioassay systems but, apart from the brain, spinal cord and retina, no positive tissues have been found (40, 81). Among those tested by intracerebral and intraperitoneal injections into mice were several reproductive tissues: semen (from three bulls), testes (one bull), uterine caruncle (one cow), placental cotyledon...
(two cows), allantoic fluid (one cow), amniotic fluid (one cow), foetal heart blood (one cow) and uterine flush fluid samples (40 cows). A thousand embryos have also been tested (20 embryos per mouse) by intracerebral injection. Details of the work involving uterine flush fluids and embryos (125) are given later. Mice have been challenged orally with placental material, and these challenges too were negative (78).

To circumvent any possible species barrier, placental material from BSE-affected cows has been tested in calves obtained from farms where meat-and-bone meal had not been fed for at least ten years. In November 1989, twelve calves were dosed with 100 ml (90 ml given orally and 10 ml intranasally) of a 50% suspension of homogenised foetal membranes, which had been taken from two natural BSE cases in their last month of gestation. Seven control calves were dosed similarly, but with water. Six of the challenged and three of the control animals were killed two years after challenge. None had histopathological lesions of BSE or 'scrapie-associated fibrils' (SAFs, see below) in the brain, and no brain infectivity was detected by mouse bioassay. The remaining six challenged and four control cattle remained healthy until they were killed seven years after challenge. Histopathological studies on the brains of these latest cattle are incomplete but all were negative for SAFs (S.A.C. Hawkins, personal communication).

The apparent lack of infectivity in lymphoid and other peripheral tissues, including placenta, of naturally affected BSE cattle is in marked contrast with the results of bioassays on peripheral tissues from sheep and goat scrapie cases. Whether this difference is due to insensitivity of the assays or to a true absence of infection in those particular tissues from BSE cattle is not yet clear. However, it is noteworthy that infectivity has been detected by mouse bioassay in the distal ileum of experimentally infected cattle at six months, ten months, 14 months and 18 months (but not at two months), after these cattle were fed a very large amount (100 g) of brain from clinical BSE cases (81, 113). Bioassays on the ileum and several other tissues taken from these cattle at later stages after the oral challenge are still in progress, but spongiform changes were found in the brains of those killed at 32 months, and clinical signs became evident at 35 months in some of those still alive (G.A.H. Wells, personal communication).

### Use of PrPSc detection methods

It is known that a close association exists between scrapie and BSE infectivity in tissues, as detected by bioassays, and the presence of PrPSc, which is demonstrable by laboratory techniques (26, 76, 102). However, whereas bioassays may take up to two years or possibly much longer, depending on the species used, it is possible to test for PrPSc relatively quickly (in a few days) by methods such as immunoblotting (Western blot) (48, 96) and immunocytochemistry (36, 79, 111). Thus, while bioassays are still needed to demonstrate infectivity, these immunological tests have obvious advantages. Immunocytochemistry has a further advantage in that it enables PrPSc to be precisely located within the infected tissue, and can show the cell types affected.

Another useful technique is the detection by electron microscopy of SAFs, which are essentially deposits of PrPSc which can be found in detergent extracts of brain and spinal cord from scrapie and BSE cases, and also in extracts of peripheral tissues such as spleen and lymph nodes from scrapie cases (104; M.J. Stack, personal communication). Deposits of PrPSc can sometimes be found in peripheral tissues of infected animals before the appearance of clinical signs, i.e. in incubating animals. This is important because it presents the possibility of taking biopsies of peripheral tissue from suspect animals for testing by electron microscopy or immunocytochemical methods.

Using immunocytochemistry, Van Keulen et al. (111) recently demonstrated the presence of PrPSc in palatine tonsil, retropharyngeal lymph node, mesenteric lymph node and spleen in a high proportion (98%) of over 50 sheep naturally affected by scrapie. PrPSc was also present in the prefemoral, prescapular and tracheo-bronchial lymph nodes of 87% to 93% of the same animals. A much higher proportion of lymphoid follicles in the tonsil contained PrPSc than did follicles in the spleen and various lymph nodes. It was for this reason, and also because the tonsil is readily accessible for biopsy, that Van Keulen et al. concluded that immunocytochemical examination of tonsil biopsies might be a good way of testing live sheep for the presence of scrapie agent. As in mice (see above), the PrPSc in these sheep tissues appeared to be associated with FDCs, but granules of PrPSc were likewise seen in phagocytic cells, presumably macrophages. However, Van Keulen et al. also found that, despite all their sheep having been confirmed as having scrapie, PrPSc could not be detected in the lymphoid tissues of one of the animals (and in another case found after the main study was completed). Both these sheep were of an unusual PrP genotype, which might explain why PrPSc was absent from their lymphoid tissues. Hadlow (51) discovered similar cases with bioassays of peripheral tissues from certain breeds of scrapie-affected sheep (e.g. the Montadale). The significance of atypical cases like this is difficult to assess, but it is evident they will reduce the sensitivity of scrapie tests that rely on tissue biopsies.

### Summary of current understanding of pathogenesis

In sheep and goats under natural conditions, it seems that scrapie infection most commonly enters the body by the oral route, although intraocular and wound contamination may also take place, and foetuses may be infected transplacentally. Following entry there is a prolonged 'eclipse phase', lasting several weeks or months, when infectivity cannot be detected anywhere in the peripheral tissues or CNS. Infectivity appears initially in the lymphoid tissues, especially those of the oropharynx (e.g. tonsil and retropharyngeal lymph nodes).
levels in these tissues tend to be close to the limits of detectability. Multiplication of the agent in peripheral tissues then continues for a variable period: a matter of months in genetically susceptible animals although in non-susceptible ones the agent may never be detected. Eventually infectivity reaches the CNS where it increases to a high level and produces clinical disease. In some breeds or genotypes the peripheral tissues remain infective throughout the CNS replication period and into the clinical phase, whereas in others (e.g. the Montadale observed by Hadlow) the only infective tissue in clinically affected cases appears to be the CNS. Whether the agent is present in other tissues but at undetectable levels, or whether it is eliminated from all but the CNS after the initial stages, is still unknown. Also unknown is whether infectivity can persist in peripheral tissues of non-susceptible animals without ever reaching and replicating in the CNS. The possibility that genetically resistant sheep might act as scrapie carriers is important because, while not succumbing themselves, they might transmit the disease to susceptible animals.

As far as BSE is concerned, although it is clear that the great majority of cattle in the British epidemic acquired infection by consumption of contaminated feed, it now seems likely that some maternal transmission has also occurred. Whether the latter takes place vertically (e.g. via the embryo or across the placenta before parturition) or horizontally after parturition is unknown. However, in contrast to scrapie in sheep and goats, bioassays for BSE infectivity in non-neural peripheral tissues of cattle (apart from in the distal ileum of cattle exposed orally to large, experimental doses of infected brain) have all been found to be negative.

How TSE agents travel within the body to reach the CNS is still unknown, but the neural and haematogenous routes are two main possibilities. Infectivity has been found in several peripheral nerves in sheep, so perhaps it travels along such nerves, as seems to be the case in mice. However, in contrast to the mouse, in which CNS infectivity is first detectable in the mid-thoracic spinal cord, in sheep and goats the infectivity appears first in the brain stem, which raises the possibility of passage along cranial nerves from replication sites in the oronasopharynx (2, 50). The alternative is that scrapie infectivity might be carried haematogenously, and this must at least be considered. While it is reasonable to accept that infection might travel from the oropharynx and other parts of the digestive tract to the CNS along neural pathways, it is less easy to understand how, in sheep and goats and perhaps in cattle, it could reach and cross the placenta without involving the haematogenous route. The potential implications of this are far-reaching because it would mean that virtually all tissues could carry infection, including (if blood contamination occurred within the genital tract) semen and embryos. Such a hypothesis also raises the possibility of TSE transmission by medical and surgical procedures. Furthermore, if the agents can occur in the blood, then it follows that serum and other animal products used in the collection and processing of semen and embryos, and in certain hormonal preparations used for superovulation, could themselves pose risks. It is emphasised that there is currently no direct evidence for any of these possibilities.

Other evidence for vertical transmission

In addition to the tissue infectivity data, there is evidence relevant to vertical transmission from demographic, epidemiological and various other studies. For example, there have been some very early cases of clinical disease and/or infection which are at least suggestive of prenatal infection. Natural cases of scrapie in sheep usually occur at between two and four years of age, and clinical onset is uncommon before 18 months (550 days) (19, 59). However, a few cases have been recorded at between 10 and 12 months (65, 126), and Sigurdarson (100) mentions cases in Iceland that were diagnosed at seven months. These are exceptionally early and probably represent the extreme lower end of the age distribution. Using the mouse bioassay, Renwick and Zlotnik (97) also demonstrated brain infectivity (as distinct from clinical disease) in an 18-week-old Border Leicester lamb which was born to a ewe which had had confirmed scrapie.

Studies on vertical transmission of scrapie, particularly in sheep, are beset with difficulty because genetic susceptibility of individuals, lines and breeds varies so greatly. Data which, at first sight, seem to point to vertical transmission of infection are on deeper investigation often found to have their real basis in susceptibility inherited from one or both parents, so the utmost care is needed to avoid misinterpretation. Some would dismiss evidence for vertical transmission of scrapie altogether (98). Nevertheless, many studies (e.g. 23) indicate that the progeny of scrapie-affected ewes have more chance of developing the disease than the progeny of affected rams, which suggests that at least some maternal transmission of infection occurs, and that inheritance is not the whole explanation. Transmission of scrapie infection from the ram cannot be dismissed entirely but, as will be seen later, there is no direct evidence for this, and transmission of a genetic predisposition is much more significant as far as the contribution of the ram is concerned (67).

Another finding relevant to maternal transmission of scrapie infection is that, in infected flocks, if lambs are removed from their dams at birth, their chances of getting the disease, although not negligible, are much reduced. Hourigan (56, 57), for example, reported that the incidence in lambs removed at birth was 10%, as compared to 16% when they were removed at four months, 29% when removed at nine months and 41% when removed at 20 months. He suggested that the raised levels of scrapie in the last three groups were due to postnatal lateral (environmental) transmission of infection.

In experimental studies, subcutaneous injection of scrapie agent into ewes just before or soon after conception has been
With regard to vertical transmission of BSE in cattle, the recently come from the interim results of a cohort experiment inversely related to the dose (1). Incubation periods (ranging from three to over five years) are varying amounts of infected bovine brain also showed that study in which cattle of known age were dosed orally with marked variation around the mean (1). Interim results from a incubation period of approximately five years, but with a in cohorts of animals of defined birth years suggests a mean one of the main difficulties experienced in studies on maternal transmission of scrapie, including studies in which foetuses were injected in utero, has been demonstrating the presence of infection in the foetuses, neonates and young offspring. This may be due in part to limitations in detection methods (e.g. the species barrier), although it is also possible that the agent exists in the ‘eclipse phase’ for some months after inoculation, especially as the tissues are immature (33). Thus, the agent may be non-infective in the usually recognised sense. However, if the reports of infectivity in ovine placenta and foetal fluids (see above) are accurate, then it is surprising that the agent has not been detected more often in foetal and neonatal lambs.

In goats, although there is evidence that scrapie can occur even when they are completely isolated from sheep (52, 58), natural caprine scrapie is rare, and there seem to be no published reports of maternal transmission in this species (121). Since it has been shown experimentally that goats, like sheep, are susceptible to BSE as well as to scrapie (34), more information on the epidemiology of natural TSEs in goats is needed.

With regard to the route of transmission, and whether calves reported to lead to very early onset of the disease in some of the lambs, e.g. at seven or eight months old (20, 47). In another experiment, direct intra-foetal injections of scrapie agent were given from half to two-thirds of the way through gestation, and tissues taken post mortem from resulting lambs at various ages up to nine months after birth were tested by mouse bioassays (54). No clinical scrapie was seen in these lambs but scrapie infectivity was detected in the lymph nodes of three of the 19 which survived to nine months after birth. In an analogous study, when goat foetuses were injected intracerebrally two months before full-term, the resultant offspring developed clinical scrapie five to seven months after birth (86). Although exceptionally early, the total incubation time in these cases was actually quite similar to that of goats injected intracerebrally three to six months after birth (88).

One of the main difficulties experienced in studies on maternal transmission of scrapie, including studies in which foetuses were injected in utero, has been demonstrating the presence of infection in the foetuses, neonates and young offspring. This may be due in part to limitations in detection methods (e.g. the species barrier), although it is also possible that the agent exists in the ‘eclipse phase’ for some months after inoculation, especially as the tissues are immature (33). Thus, the agent may be non-infective in the usually recognised sense. However, if the reports of infectivity in ovine placenta and foetal fluids (see above) are accurate, then it is surprising that the agent has not been detected more often in foetal and neonatal lambs.

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With regard to vertical transmission of BSE in cattle, the evidence from observations of very young cases of the disease is minimal. The youngest clinical case recorded so far in the British epidemic was 20 months old, and about 0.05% of all cases have been under 30 months, but the relationship of these cases, if there is any, to BSE in the dam has not been reported. If it is assumed that most cases acquired the infection soon after birth, examination of the age distribution in cohorts of animals of defined birth years suggests a mean incubation period of approximately five years, but with a marked variation around the mean (1). Interim results from a study in which cattle of known age were dosed orally with varying amounts of infected bovine brain also showed that incubation periods (ranging from three to over five years) are inversely related to the dose (1).

More direct evidence for maternal transmission of BSE has recently come from the interim results of a cohort experiment which was specifically designed to investigate the matter (1). The experiment involved monitoring 273 offspring of clinically BSE-affected cows, and 273 offspring from unaffected cows, for the occurrence of BSE. During the seven-year monitoring period, 42 offspring from the BSE group developed the disease or had histological lesions post mortem, as against 13 in the control group; a statistically significant (p < 0.0001) excess risk of 10.6% for the occurrence of BSE in the offspring of cows which had had clinical BSE themselves. The BSE cases in the control group were ascribed to the fact that offspring in both groups would almost certainly have been exposed to BSE-infected feed prior to imposition of the government ban on inclusion of ruminant protein in ruminant feed (110). All the calves in the BSE group were born within 13 months of the onset of BSE in their dams, and a large majority within five months, so the data provide little insight into the risk of maternal transmission more than six months before the onset of disease in the dam. It was assumed, however, that the risk might increase towards the end of the incubation period and in the clinical phase. With regard to the route of transmission, and whether calves were infected prenataly or in the postnatal period, the results add no information. They suggest that, although maternal transmission occurred, it may not have been true vertical transmission. Nevertheless, since most of the calves were from dairy herds, the time available for any post-parturient exposure would have been minimal; probably 24 to 48 hours at the most. It should be mentioned that, just as some (e.g. 98) have disputed the evidence for maternal transmission of scrapie in sheep, so too do they argue that these maternal transmission results for BSE are inconclusive and could have arisen from genetic differences in susceptibility between the two groups of cattle (99). Completion of the study may provide additional information.

Transmitation of scrapie and bovine spongiform encephalopathy through semen

Scrapie studies with semen and artificial insemination in sheep

Transmission of genetic susceptibility to scrapie by the ram has been thoroughly investigated (31, 59), but relatively little is known about the possibility of scrapie infection in semen, or the risks of its transmission by AI. In general, however, the role of the ram in the spread of infection is probably much less important than that of the ewe. Contact between rams and their offspring at birth, and in the months thereafter when scrapie is most often acquired, is usually minimal. In addition, as mentioned above, attempts to detect infectivity in testes, seminal vesicles and semen by mouse bioassays have all been unsuccessful. Palmer (84), in an early within-species bioassay
study, collected semen by electroejaculation from a clinically affected Suffolk ram (typical scrapie lesions were present post mortem) and injected samples of this (after dilution 1:5 in sterile water) subcutaneously into 20 one-day-old lambs of various breeds. These lambs were then observed for signs of scrapie for up to 30 months post-injection, but none developed the disease up to that time and no histological evidence of scrapie was detected in their brains post mortem. In more recent studies, it has been customary to maintain scrapie-challenged sheep for at least five years, so it is unfortunate that the observation period in this study was not longer. With hindsight, it is also unfortunate that the genetic susceptibility of the challenged lambs was unknown.

In the USA, Foote et al. (W.C. Foote, personal communication) conducted experiments involving insemination of Suffolk ewes with semen collected by electroejaculation from Suffolk rams which had been orally challenged with the SSBP-1 scrapie inoculum. The rams were held at the Mission Experimental Station, Texas, and the inseminated ewes and offspring were kept in scrapie-free premises at Utah State University. In the first (1983) experiment, pooled semen from five rams was used to inseminate 17 ewes, but no lambs were born on that occasion. Of the five inoculated rams, two developed scrapie at four months and 23 months, respectively, after semen collection. In the second (1984) experiment, pooled semen from seven rams challenged by SSBP-1 (one of which developed scrapie 14 months after collection) was used to inseminate the same group of 17 ewes, and subsequently 11 lambs were born. Four of these lambs survived for between two and five years, and seven of them for at least five years without evidence of scrapie. All 17 ewes also survived for at least five years (65 months after the 1984 inseminations) without scrapie. While these results again lend support to the view that scrapie is not transmitted through semen, it is important to note that:

- the proportions of challenged rams developing scrapie were low
- the intervals between semen collection and the onset of scrapie in these rams were long
- the semen was diluted before it was used for AI
- the Sip genotypes of sheep used in the experiments were not known
- the numbers of animals involved in the experiments were small.

Further work is obviously needed on this topic. Not only should the test semen come from clinically affected rams of a susceptible genotype, but the inseminated ewes should also be genetically susceptible and kept on scrapie-free premises for adequate observation periods.

Studies on artificial insemination in goats

There appears to be no published work on transmission of scrapie by goat semen.

Bovine spongiform encephalopathy studies involving semen and artificial insemination

As with scrapie in sheep, a number of studies have been conducted to ascertain whether susceptibility to BSE in cattle is transmitted genetically from affected sires to their offspring, but no clear evidence for this has been found so far (see above and also 15, 16). This would suggest that the risk of transmitting BSE infection through semen is also small or non-existent.

Well over 400 cases of BSE have occurred in bulls during the British epidemic, but only a small proportion have been in AI stud bulls, and most of these cases have occurred during the period of lay-off, which lasts until the bull is five or six years old, while awaiting results of progeny tests. In practice, whenever BSE is confirmed in a British bull, the official approval for AI is immediately withdrawn and any stored semen cannot then be used in the AI network. In a few cases, however, several hundred straws of semen have been obtained from such bulls for research purposes. Thus, as already mentioned, semen samples from three such clinically affected bulls have been bioassayed in mice, with negative results. Comparisons have also been made between the incidence of BSE in female offspring sired by two AI bulls which succumbed to BSE, and those of two bulls which did not develop the disease. There were a total of 81 offspring from the BSE-positive bulls and 104 from the unaffected animals. Three and four of these, respectively, developed BSE, which is not a statistically significant difference (6, 115).

As part of the embryo transfer studies carried out by the author and his colleagues (more detail of which is given below) seven-day-old, zona pellucida-intact embryos of clinically affected cows, derived by AI with semen collected from clinically affected bulls, have been bioassayed in mice with negative results. Embryos of similar status have been transferred into healthy, BSE-negative recipient heifers also. At the time of writing (November, 1996), none of these recipients (now four to five years after AI), or their offspring (now three to four-and-a-half years after birth), has shown any evidence of BSE.

Transmission of scrapie and bovine spongiform encephalopathy through embryos

Embryo transfer studies in sheep

Studies on scrapie transmission by embryo transfer in sheep have produced complex results which need careful interpretation. Host genotypes, agent strains and natural
versus experimental challenges (including the routes of challenge) are all important factors which contribute to experimental results, but control of these has not always been adequate. Moreover, because horizontal transmission of scrapie between sheep is possible, groups of embryo donors and recipients must always be strictly isolated, and rigorous precautions are necessary to avoid inadvertent transmission of infection by contaminated equipment, personnel, pastures, etc.

Dickinson, Young and Renwick were probably the first to appreciate the potential of embryo transfer for studies on scrapie transmission in sheep, and in 1964 they described a small experiment on this topic (one embryo transferred). However, no conclusions were reached (20).

In 1980, in the USA, Foote et al. commenced an ambitious scrapie study, the objectives of which were to obtain data by means of reciprocal embryo transfers between scrapie-inoculated and scrapie-free sheep on the following:

- the occurrence of vertical transmission
- the efficacy of embryo transfer for obtaining scrapie-free progeny (30).

Overall, 153 ewes and 26 rams were challenged with scrapie, but not all of these animals were used in the embryo transfer experiments. Their ages ranged from one to five years and the breeds were Cheviot and Suffolk. The Cheviots were injected subcutaneously with the SSBP-1 inoculum, whereas the Suffolks were challenged orally, subcutaneously, or by both routes, with a Suffolk-passaged scrapie strain from Texas. All the challenged sheep were kept at the Mission Experimental Station, Texas, and approximately 50% of them eventually developed clinical scrapie after incubations averaging 11 months in the Cheviots and 20 months in the Suffolks. The intended five-year observation period in the Cheviots (these comprised a third of the challenged animals) had to be curtailed by slaughter at two years, so the percentage with scrapie might otherwise have been higher.

Embryo collections from the scrapie-challenged donor ewes (mated to scrapie-challenged rams) began at less than a month after challenge in some instances, and continued for over three years after challenge in others. The intervals between collection and scrapie onset varied from zero (already showing clinical signs) to 32 months in those donors that did eventually succumb. The embryos were not frozen before transfer, and were washed three times, not ten times as later recommended in the Manual of the International Embryo Transfer Society (IETS) (105). Another IETS Manual recommendation, that embryos without an intact zona pellucida should not be transferred, was not always followed either. The embryos were transferred into a total of 198 scrapie-free recipient ewes, all of which were kept at Utah State University. Approximately half of these recipients were Suffolks and the rest were either Cheviots or Targhees (a white-faced western range breed), in roughly equal proportions. It appears that embryos from Suffolk donors were mainly transferred into Suffolk recipients, and embryos from Cheviots went mainly into Cheviots or Targhees.

Following the transfers, a total of 99 offspring were born, 32 of which died between birth and 23 months, 11 died between 24 and 60 months, and 56 died or were killed at over 60 months. The Suffolk recipients were kept under observation until death or for at least 60 months after transfer, but for most of the Cheviots and all the Targhees the interval was shorter (about two years). No clinical evidence of scrapie was seen in any of the offspring or the recipients, and histopathological examinations of brains (done on all but a few of the animals) were uniformly negative.

At first sight, the published data from Foote et al. encourage optimism that scrapie is not transmitted by embryo transfer, even when IETS Manual protocols (105) are not fully adhered to, but some aspects of the work indicate that a more guarded conclusion is appropriate. Results from his other scrapie transmission experiment, which involved transfer of embryos from scrapie-free donors into scrapie-challenged recipients, did not, as would have been expected, yield evidence for transmission of the disease in utero. The offspring in this case were removed by caesarean section at term and placed in a clean environment. Counting only those which survived for at least 60 months, and which were from recipients which actually developed scrapie following challenge, there were a total of 19 offspring, but none of these developed scrapie.

In only one of the experimental groups studied by Foote, referred to as the 'positive control group', did any cases of scrapie develop. This group consisted of 21 offspring conceived and gestated naturally, reared to weaning (at five months) by their own scrapie-inoculated mothers, and then kept under observation until at least 24, and where possible 60 months old. Two of these offspring, both from the same Cheviot ewe, succumbed to scrapie; one at 31 months and the other at 42 months. The sire, which had been challenged, and the dam succumbed to scrapie also but, of the other 19 offspring, 16 lived to 60 months or more without any evidence of scrapie.

With the benefit of hindsight, other deficiencies can be seen in these embryo transfer studies by Foote et al. The relatively low incidence of scrapie and fairly long incubation periods in the challenged animals, especially the Suffolks, suggest that variations in genetic susceptibility among the experimental sheep may have contributed to the generally negative results. Retrospective analyses of the genotypes (30, 75) revealed that, although about a quarter to a half of the Cheviots, and over half of the Suffolks, carried a PrP gene polymorphism (Gln/Gln-171) for short incubation, and thus were probably susceptible to the challenge inocula, many animals in the key groups may not have been susceptible. A further criticism is that most embryos appear to have been collected from the scrapie-inoculated donors early in the preclinical stage, i.e. soon after challenge, so reproductive tract infectivity, if it ever existed, would probably have been minimal.
In their paper (30), Foote et al. mention a separate study in which they were transferring embryos from Suffolk ewes with natural (clinical) scrapie into scrapie-free Targhee recipients to produce a small number of offspring. Methods, including the three embryo washes, and two separate premises, were essentially the same as in the main experiment. Between 1988 and 1994, it appears that approximately 28 offspring were born whose dams had been confirmed scrapie-positive and none of these offspring or their surrogate dams have so far developed clinical scrapie (G.R. Holyoak, personal communication). Some of the offspring, having reached 60 months of age, have been examined post mortem for scrapie lesions, and for PrP SC in the brain by immunohistochemistry and immunoblotting, and all gave negative results. Biopsies of mesenteric lymph nodes, taken from the younger offspring when approximately three years old, are also being tested for PrP SC and, so far, these too have given negative results. Overall, therefore, and bearing in mind that their latest work is incomplete, the studies by Foote, Holyoak and colleagues do give grounds for optimism that embryo transfers from scrapie-infected sheep may be safe.

The other team using embryo transfers to study transmission of scrapie in sheep is led by Foster at the NPU in Edinburgh, Scotland. It is important to note that all the animals in these studies came from the long-established NPU experimental flock of Cheviot sheep, and information on Sip and PrP genotypes obtained by PCR and RFLP was incorporated into the design of the experiments. Foster et al. referred to their first experiment (33), which started in 1988, as a 'worst-case scenario' because not only were none of the embryos washed prior to transfer but the embryo donors were of susceptible genotypes and had previously been challenged with the highly virulent SSBP-1 inoculum. Six donor ewes, two of sAsA and four of sApA genotype, were used, together with a ram that was of sApA genotype. The ewes were not inoculated subcutaneously six months prior to embryo collection, and clinical signs of scrapie appeared in all of them between six weeks and six months after collection. The ram was not inoculated until after his semen had been collected for AI but, 11 months after challenge with SSBP-1, he too developed scrapie and was killed. A total of 37 embryos were transferred into 16 recipient ewes, 15 of which were genotype pApA and one sApA. These recipients were aged three to five years at the time of transfer and, while six of them had to be culled fairly early for reasons unrelated to scrapie, nine were still healthy three years after transfer, and one (the sApA genotype) was almost eight years old when killed due to old age.

From the 16 recipients a total of 26 embryo transfer offspring were born, but six died within a year from causes unrelated to scrapie. Of the remaining 20, three were found to be of pApA genotype, 11 were of sApA genotype, and six were sAsA. Of the last-mentioned, five developed scrapie and were killed at just over two years of age (751-783 days), and the sixth developed the disease about seven months later (979 days). These cases were confirmed positive for scrapie by brain histopathology, by electron microscopy for SAFs and/or by immunoblotting for PrP SC. At the time of the first paper of Foster et al. (33), all of the remaining offspring were still healthy, but in their next publication (35), they reported that two of the sApA offspring had subsequently developed scrapie at 1,006 and 1,270 days. Moreover, a further six of the nine sApA genotype offspring had to be killed due to metabolic illness, and two of these, killed at 988 and 1,013 days, although not having clinical signs or histopathological lesions of scrapie, were found to be positive for PrP SC by immunoblotting.

At this stage of these studies, with half the surviving embryo transfer offspring giving positive results for scrapie at relatively early ages, many questions were being raised about the work. Some, for example, Ridley and Baker (98), doubted whether maternal transmission of infection had occurred and favoured instead a genetic explanation, with the disease having arisen de novo in the highly susceptible genotypes. Others suggested that scrapie transmission may have occurred due to lack of washing of the embryos, or postulated that perhaps the resistant (Sip pApA) recipients were subclinically infected and had infected the offspring in utero. Environmental contamination after birth in the recipient flock was another possibility. The authors acknowledged these questions (35) and began further transfers from infected and uninfected (control) ewes. This time some embryos were washed according to IETS protocols, while others were left unwashed.

The most recent studies of Foster et al. were reported in June, 1996 (37). As before, all their sheep were from the NPU Cheviot flock, but, in addition to Sip genotypes (primarily linked with polymorphisms at PrP codon 136; see above), data on PrP codons 154 and 171 were also known (63). Two groups of embryo donors were used. The first consisted of three unchallenged ewes of Sip sApA genotype to provide control embryos, and the second group consisted of three sApA and three pApA ewes, which were inoculated subcutaneously with the SSBP-1 inoculum to provide potentially infected embryos. The challenged donors were inoculated about eight months prior to embryo collection and the collections took place at 60 to 100 days before scrapie onset in the sApA donors. As expected, the three challenged pApA donors did not develop the disease (approximately five years after inoculation). Two sAsA rams were used to provide semen for AI and, although neither was challenged, both developed scrapie naturally when they were about two years old, which was approximately eight months after semen collection.

All the recipient ewes were of pApA genotype and over five years old when the embryos were transferred, and although none developed clinical signs or had evidence of scrapie post mortem, their observation periods were fairly short. Strict precautions were taken to try to ensure that the embryo transfer media, equipment and operating theatre would not pose scrapie risks to the recipients or to the embryos at time of...
transfer. In addition, at lambing and during rearing, efforts were made through disinfection (with 20% sodium hypochlorite), group segregation and control of husbandry procedures to avoid the entry of extraneous infectivity. However, while the groups were kept in separate paddocks on re-seeded pasture that had not previously been used for parturient or experimental sheep, there appear to have been no great distances between them, and this aspect of the experimental protocol might be open to criticism.

A total of 28 offspring were born and 10 of these had developed scrapie by the time the next paper by Foster et al. was written (37). Numbers in the different groups, with the Sip genotypes and ages at death by scrapie, are shown in Table II, which is adapted from that paper.

Rather than providing clear answers to questions arising from their first experiment, these results from the second have prompted even more questions! Several (though not all) of the sAsA offspring developed scrapie and most did so at similar ages to those in the earlier study, i.e. between two and three years. The origin of these offspring, i.e. whether from the unchallenged (control) donors or the challenged donors, seems to have had no clear effect on the scrapie rate or age at death. Embryo washing made little difference either. Nevertheless, as Foster et al. point out, three embryo transfer offspring of sAsA genotype survived for well over four years (now almost five years; J.D. Foster, personal communication), which, they say, is very significant because sAsA sheep in the herd never developed scrapie by the time the next paper by Foster et al. was written (37). Numbers in the different groups, with the Sip genotypes and ages at death by scrapie, are shown in Table II, which is adapted from that paper.

With regard to the 15 Sip sApA offspring, it is evident from Table II that, apart from the two intercurrent deaths, all of these survived for at least four years. However, in the discussion of this study (37), and also in that of a parallel paper from the Edinburgh NPU team (63), Foster et al. comment that more detailed analyses of the Sip/PrP genotypes of these particular animals revealed that 'only one is liable to be susceptible to natural scrapie'. This is interesting since it indicates their suspicion that most, if not all, of the scrapie cases among the embryo transfer offspring were of natural origin, rather than being transmitted from the SSBP-1 inoculated donors. They also state ‘... it remains possible that the progeny from the embryo transfers were infected at or around the time of lambing, despite the stringent precautions taken to prevent it. Lambs may be particularly susceptible to infection from scrapie in the environment at this time.’ This could explain why some of the sAsA genotype offspring derived from the unchallenged donors also developed the disease.

Probably the most compelling evidence pointing to natural (environmental) scrapie rather than the SSBP-1 inoculations being responsible for the cases in the embryo transfer offspring is that the clinical manifestations and histological brain lesions in most of these offspring bore a close resemblance to those of the natural type of scrapie endemic in the NPU Cheviot flock, rather than to the distinctive changes known to be produced by SSBP-1 inoculum. The development of natural scrapie in the two sAsA rams eight months after their semen was used to sire the embryos also raises the possibility, albeit remote, of paternal infection of the offspring. The experiment appears to shed little new light on whether scrapie infectivity can be passed in utero to offspring as a result of the (hypothetical) carrier state in resistant genotypes. However, prolonged survival of three of the Sip sAsA offspring shows that natural scrapie does not necessarily always arise spontaneously in highly susceptible genotypes, as some have proposed (98).

In conclusion, despite the considerable research already performed in both the USA and Scotland, the question of whether scrapie may be transmitted by embryos remains unanswered. In future work it would be preferable to obtain some control cases among the embryo transfer offspring were of natural origin, rather than being transmitted from the SSBP-1 inoculated donors. They also state ‘... it remains possible that the progeny from the embryo transfers were infected at or around the time of lambing, despite the stringent precautions taken to prevent it. Lambs may be particularly susceptible to infection from scrapie in the environment at this time.’ This could explain why some of the sAsA genotype offspring derived from the unchallenged donors also developed the disease.

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### Table II

<table>
<thead>
<tr>
<th>Offspring type</th>
<th>Donors unchallenged sApA</th>
<th>Donors challenged sApA</th>
<th>Donors challenged pApA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number born</td>
<td>Age at death (days)</td>
<td>Number born</td>
</tr>
<tr>
<td>sAsA washed</td>
<td>4</td>
<td>866, 1,000, two survivors</td>
<td>3</td>
</tr>
<tr>
<td>sAsA unwashed</td>
<td>4</td>
<td>778, 866, 888, 888</td>
<td>2</td>
</tr>
<tr>
<td>sApA washed</td>
<td>3</td>
<td>Three survivors</td>
<td>1</td>
</tr>
<tr>
<td>sApA unwashed</td>
<td>4</td>
<td>Four survivors</td>
<td>2</td>
</tr>
</tbody>
</table>
possible sources of extraneous infection, including contaminated pastures, medicinal products and feed. The recent report from Iceland that hay mites may be responsible for carriage of scrapie infectivity (120) is a salutary reminder of the extreme caution needed in these respects.

**Embryo transfer studies in goats**

Although little has been formally published about their work (29), some embryo transfers have been performed in goats by Foote et al. in the USA to investigate scrapie transmission (G.R. Holyoak and W. Foote, personal communication). Methods were generally similar to those used in their work with sheep. Donors (Spanish breed) were challenged orally with the Suffolk-passaged scrapie inoculum before embryo collection, and were later confirmed to be positive for scrapie by brain histology. None of the embryo transfer offspring or their surrogate mothers developed scrapie during five-year observation periods.

Foster et al. have reported embryo transfers from donor goats which had been challenged subcutaneously or intracerebrally with BSE 13 months prior to embryo collection (35). A total of 10 of the 11 donors developed scrapie-like symptoms between 18 and 42 months after challenge. Transfers of the embryos into 29 recipient nannies, and their subsequent kidding, took place under hygienic conditions similar to those used in the NPU sheep scrapie experiments, and the offspring were segregated from other livestock on pasture. There was a total of 37 offspring and, although some died or were killed due to intercurrent disease, the rest remained healthy and without signs of scrapie for up to five years after birth (J.D. Foster, personal communication).

**Embryo transfer studies on bovine spongiform encephalopathy in cattle**

An experiment designed to show that embryos from BSE-affected cattle do not transmit the disease is in progress in the UK. Reports on the initial phase of this work, which began in 1990, have been published before (5, 122, 123, 124), but an update is given here.

Two hundred cows, field cases suspected on clinical grounds of having BSE, were brought to the Central Veterinary Laboratory in southern England to serve as embryo donors. They were superovulated, inseminated by AI and flushed non-surgically to collect the embryos at seven days. Semen from eight confirmed BSE-positive bulls was used for approximately half the inseminations and semen from five healthy bulls collected before 1981 (at least five years before the first known case of BSE) was used for the remainder. The deteriorating clinical condition of some donors precluded repeat collections but most were flushed to collect further embryos at approximately monthly intervals until euthanasia. The average number of flushes was 2.9 but a few donors were flushed six times over a period of six months. All embryos were frozen and stored pending transfer. Prior to freezing, they were screened microscopically to ensure an intact, clean zona pellucida, and washed ten times as recommended in the IETS Manual (105). Bovine serum in the media used for collection, washing, storage and transfer of the embryos was obtained from TSE-free sources (New Zealand), and strict precautions were taken to avoid the possibility of TSE contamination through equipment, personnel, etc.

Before any of the embryos were used, brains of the donor cows were examined histologically postmortem to confirm the diagnosis of BSE, and all the embryos from 33 donors found to be BSE-negative were excluded from the experiment. Unfertilised and poor-quality embryos, and also samples of uterine flushing fluid, were bioassayed for infectivity in mice, but the good (transfer quality) embryos were transferred non-surgically into recipient heifers.

Bioassay of poor-quality embryos and flushing fluids was conducted in collaboration with the Institute for Animal Health, Compton, and the NPU, Edinburgh (125). The embryos for bioassay were sonicated and injected intracerebrally into 51 mice of known susceptible Sinc genotypes, each mouse receiving a 0.02 ml suspension containing 20 embryos. Roughly half the mice received embryos derived from BSE-positive cows and BSE-positive bulls (termed +/+ embryos), and the other half received +/- embryos, i.e. from positive cows and negative bulls. The uterine flush fluid samples were also of the two types: 17 were +/+ and 23 were +/- A total of 1,000 mice were used for the bioassays of flush fluid, each mouse being injected both intracerebrally (0.02 ml) and intraperitoneally (0.10 ml). Following injection, mice were kept under observation for up to 700 days and brain examinations were performed on all those dying or killed, for whatever reason. All but one of the mice were negative, and the one which did not give negative results, which had had +/- flush fluid, was inconclusive, although other evidence, including its age at death, suggested that it too was probably negative. Overall, therefore, our mouse bioassay results indicate that infectivity in the embryos and flush fluids was absent or at least undetectable, which is encouraging. Nevertheless, because of the species barrier effect for BSE, a possibility remains that the mouse bioassay was insufficiently sensitive to detect any BSE infectivity in the embryos and fluids, so it would be prudent to wait for the results of embryo transfers into cattle.

The embryo recipients consisted of Hereford × Friesian heifers from New Zealand, which were imported by air to the UK in 1990 (132 heifers) and 1991 (220 heifers). New Zealand was the chosen source because it is free from scrapie and BSE and has a sound livestock TSE surveillance system. Among other criteria used in selecting recipients (and some Limousin bulls for subsequent breedings) was the fact that, for several years at least, their herds of origin had imported no ruminants, ruminant semen or embryos from the UK. Since arrival, the cattle have been kept under strict security as a closed beef suckler herd on an upland farm in northern England.
Although clinical cases of BSE had never occurred on this farm, it was nevertheless depopulated of all livestock, and the buildings and equipment were disinfected with 20% sodium hypochlorite. A strict code of practice is in force to prevent the possible introduction of scrapie or BSE infection from extraneous sources. For example, no cattle vaccines are used, and all feedstuffs (grass, silage, straw, cereals) are home-grown or sourced from selected non-livestock farms.

Transfers of embryos took place in three sessions: July/August 1991; July/August 1992 and November/December 1992, and all but seven of the 352 imported heifers were used as recipients. Some recipients that returned to oestrus after transfer had further transfers in the same or (in 1992) subsequent session. A total of 266 liveborn offspring were produced and, of these, 53% had a BSE-positive sire as well as a BSE-positive dam.

The offspring are being kept for seven years (from date of birth) and recipients likewise (from date of first embryo transfer), after which time all survivors will be killed and examined for BSE. In the interim, they are being monitored clinically for BSE and any that die undergo examination post mortem, including brain histology. So far (November 1996), 11 recipients and 11 of the embryo transfer offspring have died, but no evidence of BSE, clinical or otherwise, has been found. Planned slaughter and examination of the recipients for BSE is scheduled from July 1998 to December 1999, and that of the offspring from March 1999 to October 2000. It is too early, therefore, to speculate about the final outcome of this experiment. Nevertheless, the absence of any evidence of BSE at this stage, more than five years after the first transfers, is encouraging.

Reducing the risks of scrapie and bovine spongiform encephalopathy transmission

Sanitary precautions for conventional diseases

It is well known that if appropriate sanitary precautions are taken, the risks of semen and embryos carrying conventional infectious diseases are very small (see other papers in this issue). The risks with embryos tend to be lower than for semen, but they are not totally absent and should not be ignored. In addition, the costs and complexity of using embryos tend to be higher than for semen. The possibility of transmitting disease susceptibility genetically via semen and embryos is a special risk factor associated with these commodities, and one which is particularly relevant to sheep scrapie and perhaps other TSEs. In this connection it should be remembered that, whereas the spermatozoa contain only half the genetic complement of the individual, embryos contain the full complement. Thus, although embryos have advantages for genetic improvement of productivity traits, when it comes to inherited susceptibilities to disease, transmission of the full genetic complement may lead to increased risks. Conversely, genotyped embryos and semen may be used to increase resistance to diseases such as scrapie.

Sanitary procedures for AI are set out in the International Animal Health Code of the OIE (83) which emphasises the need for semen to be collected and processed under veterinary supervision in officially approved AI Centres. Equivalent procedures for embryos are also set out in the International Animal Health Code (83) and in the Manual of the IETS (105), and these again emphasise that the work should be done under veterinary supervision, by officially approved embryo collection teams. In essence, provided an embryo has an intact zona pellucida and is subjected to a defined regime of ten washings, it can be transferred with a high degree of safety even if the donor was infected. Washing, or washing in conjunction with trypsin treatment, is extremely effective for removing a variety of pathogens, although it needs to be said that no specific research on this has been conducted with TSE agents.

The ability to cryopreserve semen and embryos of cattle, sheep and goats for long periods without seriously affecting their viability provides important additional safety margins with regard to disease transmission. Thus, while they are held frozen in storage prior to export, post-collection surveillance and in some cases tests on donors, can be performed to ensure that specified diseases had not been incubating at the time of collection.

Sanitary procedures apply not only to the selection and health testing of the donors but also to the choice and use of equipment and media, particularly where the latter contain substances of animal origin (e.g. bovine serum). With regard to scrapie and BSE, the equipment and media are especially crucial; extreme resistance of TSE agents means that traditional procedures used to inactivate micro-organisms are totally inadequate for these (107, 108). Thus, if TSE transmission risks are perceived as a threat, equipment must be of the single-use, disposable type, or be autoclaved to stringent TSE standards. There is now general agreement that biological substances in media and in other products used for embryo collection, processing and transfer should be derived from animals in scrapie- and BSE-free countries or herds (14, 114).

Balancing the risks and benefits of importation

Methods for managing and reducing the risks of importation of most conventional diseases are sophisticated and usually reliable. However, because scrapie and BSE are in many respects unconventional, risk reduction methods such as serological testing, clinical surveillance, movement restrictions and quarantine are either inappropriate or require substantial enhancement. Basic problems are the extremely long incubation periods and inability to identify animals incubating disease or carrying infection. Such animals might
be shedding the agent and thus posing a threat to other animals long before clinical signs develop. Testing lymphoid tissue (e.g., tonsil or mesenteric lymph node) biopsies appears to have potential for detecting infectivity in live sheep, but this is a cumbersome method and its sensitivity over a range of scrapie strains in sheep of different PrP genotypes is unproven. Immunological testing for PrPSc in tissues is much quicker than bioassay, and is useful if samples are positive, but this does not necessarily provide information about infectivity. Prolonged quarantine of the embryo recipients and offspring on, for example, a remote island is another option, but this is very expensive. In view of all the special problems with TSEs, pessimistic attitudes and ‘zero risk’ policies against the importation of genetic material are understandable. However, such policies are not always justified because, with careful management, the risks can be reduced to extremely low levels without a total ban on movement.

Attitudes to importation depend not only on the risks of introducing diseases but also on whether the diseases in question are already present in the importing country, and on the priority attached to procurement of new bloodlines for livestock improvement. For example, one or more animal TSEs may already be present, but import restrictions might be prompted by a desire to avoid the introduction of new TSE strains. On the other hand, countries free of TSEs in their livestock may be ultra-cautious about importation risks, to avoid jeopardising their own livestock industry and export trade. As pointed out by MacDiarmid (74), the absence of TSEs in New Zealand and Australia has resulted in a world-wide demand for animal products from those countries for use in the biopharmaceutical industries. Similarly, with regard to defined regions within countries, or even individual flocks and herds which have been effectively monitored and certified free of scrapie and/or BSE, it may be possible to sell animals and animal products. Cautious importation strategies tend, however, to be counterproductive where rapid improvement in livestock productivity is an objective, because those pursuing this aim will often require unlimited choice of breeding animals. Herein also lies a dilemma for sheep breeders in scrapie-endemic countries or regions who wish to select for resistant PrP genotypes, because by doing this they may complicate or hinder genetic selection for other desirable parameters. It should be emphasised that measures to protect against the introduction of TSEs via animals, semen and embryos must also cover the potential risks of TSE infectivity in other materials, such as animal proteins, vaccines and vectors (e.g., mites).

Reducing possible risks of scrapie transmission by sheep semen and embryos

Where absence of scrapie from particular flocks or countries can be guaranteed, obtaining risk-free semen or embryos from them is relatively straightforward. However, apart from a few recognised countries with long-standing freedom from scrapie, such guarantees are difficult to obtain. As pointed out by Detwiler (17), it is almost impossible to determine the true incidence of scrapie in endemic countries, especially where economic factors influence the willingness of flock owners to report cases. For these reasons, therefore, and because testing of healthy donors for infectivity is at best unreliable, it is necessary to rely on risk reduction measures. As far as AI and embryo transfer are concerned, current research indicates that these should be regarded as measures that will substantially reduce but not wholly eliminate risks. Thus, depending on the scrapie status and aspirations of the importing country, region or flock, a package consisting of some or all of the following precautions should be used:

- increase chances that the offspring will be resistant to the most common scrapie strains, or
- increase chances that clinical manifestations of scrapie, if they occur, will do so early, while the offspring are in quarantine
- ensure that the sanitary protocols for preventing transmission of conventional pathogens via semen and embryos, as set out in Appendices 4.2.2.2. and 4.2.3.3. of the OIE International Animal Health Code (83), are followed
- use only frozen semen and embryos so these can, if required, be stored pending post-collection surveillance or testing of donors, if this is deemed necessary
- take biopsies of tonsil or lymph nodes (or a wider range of tissues, including brain, if the donors are killed at collection) and test these for PrPSc by immunological methods, and/or for infectivity by bioassays using genetically susceptible sheep, goats or mice
- select recipients with appropriate PrP genotypes for either resistance or susceptibility (depending on the circumstances; see b) above) and transfer the semen or embryos into these recipients
- use an appropriately isolated quarantine station (e.g., a remote island) which is under official veterinary supervision
- keep the offspring and recipients in quarantine under observation for clinical scrapie for at least five years, and conduct post mortem examinations for scrapie on all those that die
- pursue a breeding programme in the quarantine to produce cryopreserved semen and/or embryos, and second-generation progeny, and keep these progeny under observation also
- slaughter all surviving first-generation offspring and the recipients after five years and perform full post-mortem examinations, including tests for PrPSc and bioassays for infectivity
k) on satisfactory completion of the quarantine programme, release second and subsequent generations, plus any semen and embryos, to their destined mainland flocks, but ensure these flocks are registered and kept under official supervision for at least a further five years.

Obviously, the use of all these risk reduction measures would be appropriate only in the most extreme circumstances, for example, importations from scrapie-endemic countries into countries with large, scrapie-free sheep populations. Otherwise, an appropriate package of measures should be selected which reduces the risk to an acceptable and cost-effective level. Decisions on the appropriate risk management package should be based on quantitative risk assessment as proposed for scrapie by MacDiarmid (74), and for other diseases by Sutmoller and Wrathall (106). In the case of conventional flocks of unknown scrapie status, which merely need to obtain new bloodlines, it is probably best to import semen or embryos from flocks in the same region. If donors with resistant genotypes can be identified, these should be used, despite the fact there is no guarantee they will resist all scrapie strains. Other precautions listed above are unlikely to be economical or practical for movements between flocks. Where bioassays on biopsies or on post mortem tissues are applied for risk reduction, it should be remembered that their sensitivity is reduced by the species barrier. Thus, bioassays in sheep and goats, although more expensive if adequate numbers are used, are better than those in mice. If future research shows convincingly that AI and/or embryo transfer can be relied on to reduce the risks of scrapie transmission to negligible levels, this will be of enormous benefit to trade. In these circumstances, the need for other precautions would be drastically curtailed.

Reducing possible risks of scrapie transmission by goat semen and embryos

The information on scrapie transmission risks for goat semen and embryos, although limited and mostly unpublished, gives grounds for optimism that the risks are extremely small or non-existent. The apparent lack of major genetic influences on scrapie in goats is also encouraging. Nevertheless, because the introduction of scrapie into a previously scrapie-free country might have very serious consequences, it is essential to apply at least some of the risk reduction measures recommended for sheep when importing goat semen and embryos.

Reducing possible risks of bovine spongiform encephalopathy transmission by semen and embryos

The threat posed to countries and livestock producers by BSE differs in some important respects from the threat of scrapie, and risk reduction methods are also different. For example, relatively few countries have reported any cases of the disease, and only in the UK, where over 165,000 cattle have died during the current epidemic, is BSE perceived as a major livestock disease problem. Another difference is that, whereas scrapie seems to be transmitted both vertically and horizontally, and tends to become endemic in populations and flocks, BSE is not thought to be contagious in that way. However, unlike scrapie, which is not regarded as a zoonosis, it is thought that BSE could be transmissible to humans and may have been responsible for some cases of CJD of an unusual type ('new variant' CJD) recently reported in the UK (117). Consequently, stringent measures are being taken by most BSE-free countries to prevent entry of the disease, and those countries with BSE are striving to eliminate it.

At the General Session of the OIE in Paris in May 1996, a revised chapter, Chapter 3.2.13., was written for the International Animal Health Code (83), setting out recommendations for the importation of cattle and cattle products from BSE-affected countries. Countries are placed into three categories: those with a high incidence of BSE, those with a low incidence, and those that can be considered free of the disease. The official BSE status of a country must be determined by continuous surveillance and monitoring, which includes the compulsory notification, isolation and clinical investigation of all suspected cases. Brains of clinical suspects which are slaughtered or die must be properly examined, and the numbers of investigations and confirmed BSE cases must be recorded. Countries may be considered free of BSE if no cases of the disease have occurred at all, or if any cases that have occurred originated from the importation of live cattle from BSE-affected countries. Obviously, any such cases must be slaughtered and completely destroyed, and the feeding of ruminants with meat-and-bone meal derived from other ruminants must be banned and effectively enforced.

In the absence of scientific evidence as to any risk associated with semen, recommendations are not made about this in the BSE chapter of the OIE International Animal Health Code (83). Surprisingly, however, since evidence about any BSE transmission risk from embryos is also still lacking, recommendations about embryo trading are given. These state that embryos to be imported from countries with a low incidence of BSE should be derived from animals not clinically affected with BSE, or from their daughters. In addition, the chapter recommends that, for embryo importations from countries with a high incidence of the disease, the donors should have been born after the date on which an effective ban on the use of ruminant meat-and-bone meal in feed for ruminants came into force. Alternatively, the donors should have been born, raised and remained resident in a herd in which no case of BSE had ever been confirmed, and they should never have been fed ruminant meat-and-bone meal. It is to be hoped that the embryo transfer experiments currently in progress in cattle in the UK, when completed, will give reassurance as to the low risks of BSE transmission by this route.

Chapter 3.2.13. of the International Animal Health Code (83) also refers to the need for careful selection and avoidance of contamination of materials of bovine origin used in the
manufacture of medicinal products. Since this applies to animal products used in AI and embryo transfer techniques, it is important to draw attention to it. Examples include the use of bovine serum in embryo transfer media and the use of follicle-stimulating hormone (FSH) for superovulation. In view of its anatomical site of origin, pituitary FSH carries a particularly high risk of carriage of TSE agents, so great care must be taken with its source and preparation (66). In addition to the specific measures needed to avoid risks of transmitting TSEs through semen and embryos in cattle, it is essential to follow the general sanitary procedures recommended for bovine semen and embryos in the appropriate Appendices of the International Animal Health Code (83).

Concluding remarks

To quote from Detwiler (17), '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.' Most of these features characterise BSE as well, although in other ways BSE is different from scrapie; for example, it seems to be less contagious, host genetic factors are less important, and only one strain of TSE agent appears to be involved. Whatever their similarities and differences, one thing is clear, and that is that control of both diseases has a high priority. In addition, if control is to be achieved without loss of valuable livestock gene pools, AI and embryo transfer have important roles to play. As this paper shows, there is a large amount of research pointing to the efficacy of these techniques for preventing disease transmission. Admittedly, some of the sheep data seem at first sight to suggest that scrapie may be transmitted by embryos, but actually, when analysed carefully, they suggest a lack of transmission by this route and, on balance, probably strengthen the case for the safety of embryo transfer more than they weaken it. Undoubtedly, more work is needed to clarify this particular aspect, but if it can be confirmed that semen and embryos are indeed very low-risk commodities, so far as scrapie and BSE are concerned, this will give renewed hope for successful control programmes. It will also encourage greater use of AI and embryo transfer for the movement of genetic material between countries, regions and farms.

Acknowledgements

The author thanks R. Bradley, M. Dawson, J.D. Foster, S.A.C. Hawkins, G.R. Holyoak, I.M. Parsonson, M.J. Stack, G.A.H. Wells and J.W. Wilesmith for helpful discussions and/or permission to mention unpublished work. He is also grateful to his colleagues in the Import/Export Committee of the International Embryo Transfer Society for their help and encouragement. The opinions expressed in this paper are not necessarily shared by these people, nor do they necessarily reflect the views of the Central Veterinary Laboratory or any other (British) government department.
résultats de la deuxième équipe montrent que les embryons, même après lavage, peuvent être porteurs de l'agent pathogène et contaminer la descendance. S’agissant des caprins, même si des différences génétiques existent en termes de sensibilité, elles sont bien moins évidentes que chez les ovins. Il n'y a aucune preuve de transmission verticale ni par la semence ni par les embryons.

Concernant la BSE, il semble qu'il n'y ait pas ou presque pas de différences génétiques en matière de sensibilité; des travaux récents montrent, cependant, que la maladie peut se transmettre de la vache au veau. La voie de transmission et le stade, ou les stades, auxquels se produit cette transmission restent néanmoins à élucider.

En conclusion, même s'il existe de plus en plus de preuves que la tremblante et la BSE ne se transmettent probablement pas par la semence et les embryons, les recherches doivent être poursuivies pour en avoir confirmation. En attendant, il convient d'envisager des mesures de réduction des risques, surtout lorsque les semences et embryons sont importés dans des pays indemnes de ces maladies.

**Mots-clés**

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**Riesgos de transmisión del prurigo lumbar y de la encefalopatía espongiforme bovina a través del semen y de embriones**

A.E. Wrathall

**Resumen**
El autor examina el estado actual de los conocimientos sobre la transmisión del prurigo lumbar y de la encefalopatía espongiforme bovina (bovine spongiform encephalopathy: BSE) a través del semen y de embriones. Resulta difícil, especialmente en el ganado lanar, distinguir entre la transmisión genética de la predisposición al prurigo lumbar y la transmisión vertical de la infección. Sin embargo, la transmisión vertical ha sido comprobada, y se piensa que ocurre por vía placentaria; la hipótesis de un riesgo de transmisión del prurigo lumbar a través del semen no parece fundada.

Dos equipos han emprendido el estudio de la transmisión del prurigo lumbar a partir de lanares experimentalmente infectados utilizando la transferencia de embriones. Aunque el primer equipo pudo poner en evidencia la transmisión, los resultados obtenidos por el otro equipo parecen indicar que los embriones, incluso tras un lavado, pueden ser portadores del agente y transmitirlo a la progenie.

En lo que concierne a la cabra, y pese a que existen diferencias genéticas en cuanto a la sensibilidad, éstas resultan mucho menos obvias que en la oveja. La existencia de transmisión vertical o a través de semen o embriones no ha podido ser comprobada.

Respecto a la BSE, y aunque las diferencias de predisposición genética parecen inexistentes o insignificantes, algunas investigaciones recientes sugieren que tal vez se produzca la transmisión de la enfermedad de la vaca al ternero. Sin embargo, el modo de transmisión y la o las etapas en las que se produce el contagio son todavía desconocidos.
En conclusión, y pese a la certidumbre cada vez mayor de que es muy improbable que el prurigo lumbar y la BSE se transmitan por el semen y los embriones, se requieren más investigaciones para confirmar esta suposición. Por otra parte, y hasta que toda posibilidad de riesgo quede descartada, deben adoptarse las medidas de reducción de los riesgos que se consideren necesarias, especialmente en el caso de importación de semen o embriones a países libres de la enfermedad.

**Palabras clave**
- Análisis de riesgos
- Comercio de ganado
- Encefalopatía espongiforme bovina
- Enfermedades causadas por priones
- Inseminación artificial
- Patogénesis
- Prurigo lumbar
- Transferencia de embriones
- Transmisión de enfermedades.

**References**


