

# Bovine spongiform encephalopathy in sheep?

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## Summary

Bovine spongiform encephalopathy (BSE) in sheep has not been identified under natural conditions at the time of writing and remains a hypothetical issue. However, rumours about the possible finding of a BSE-like isolate in sheep have led to great unrest within the sheep industry, among the general public and within governmental and regulatory bodies. The difficulties of implementing a proper risk assessment and pre-emptive measures, in the absence of a confirmed case, are described.

The authors attempt to list what is known about experimental BSE in sheep, the distribution of infectivity in the host, some aspects of risk assessment and management and the most promising methods for differentiating BSE from scrapie in the same host. As for the latter, new and promising methods are being developed and appear suitable for initial screening of isolates of transmissible spongiform encephalopathies, but in the absence of proper validation, use of the 'classical' strain-typing in a mouse panel is still indicated.

## Keywords

Bovine spongiform encephalopathy – Cleavage – Differentiation – Experimental transmission – Glycosylation – Review – Risk assessment – Scrapie – Sheep – Strain-typing.

## Introduction and background

Ever since results concerning the successful experimental transmission of bovine spongiform encephalopathy (BSE) into sheep were published (22), the possibility that BSE could be present in small ruminants, and consequently pose a risk for consumers, has been a concern of the research community. Indeed, small ruminants had been exposed to possibly infected meat-and-bone meal (MBM) in the same way as their larger relatives. However, policy makers realised the potential risk associated with BSE in sheep only several years later. This was enhanced by the events of 1996 when the likelihood of a link between BSE in cattle and the occurrence of a new variant of Creutzfeldt-Jakob disease (vCJD) in humans (69) was officially recognised by the Government of the United Kingdom (UK).

Almost immediately after this, the World Health Organization (WHO) recommended that 'no part or product of any animal which has shown signs of a transmissible spongiform encephalopathy (TSE) should enter any food chain, whether human or animal' (71). On hindsight, this recommendation

appeared almost prophetic and was at least highly assertive for an international body. However, on closer inspection of the full text of the document, no reference is made to the risk and zoonotic potential of BSE in sheep, with only cattle being considered. Furthermore, the preceding meeting of the same Expert Committee had not pointed at BSE in sheep as being an important potential risk factor (70), and only referred to the classical forms of scrapie. The implication of the WHO recommendation was that scrapie was more 'suspect' as being able to infer a risk for consumers, which had not been the intention of the Expert Committee (F-X. Meslin, personal communication, 1999).

Since around 1998, numerous advisory and policy preparing bodies have considered the potential of BSE in sheep as a possible risk for humans and a threat to the livestock production system. The authors have made ample use of the sometimes very thorough work performed by these groups and committees, both authors having either been members or contributed to the outcome of several of the groups. These bodies often met in the aftermath of publications that sometimes bore ominous titles. One of the first examples of

these was the paper entitled 'Similar signature of the prion protein in natural sheep scrapie and BSE-related diseases' (1). Others were contained in media statements, such as that from Prusiner and Scott about the possible existence of a BSE strain in Suffolk sheep with scrapie (41). Unfortunately, no subsequent scientific publication substantiated the original newspaper article (56).

Another such media statement was made in the autumn of 2001 concerning a pool of almost 3,000 brains from scrapie-affected sheep, collected for an earlier rendering study (64) and that were thought to offer an opportunity to search for sheep BSE. However, deoxyribonucleic acid (DNA) analysis of the pool, to test for possible bovine brain contamination, revealed that the pool did indeed contain cattle as well as sheep tissue. Unfortunately, extensive public discussion surrounded the experiment, even before full results became available. Nevertheless, these events led to better understanding of the UK Contingency Plan (10) which seriously discussed a plan to depopulate the British Islands of sheep as one option.

Among the advisory bodies commenting upon the issue of BSE in sheep were the European Commission (EC) Scientific Steering Committee (SSC) and their working groups, the British Spongiform Encephalopathy Advisory Committee (SEAC) and the French counterpart, the Agence Française de Sécurité Sanitaire des Aliments (AFSSA), as well as the WHO, the Food and Agriculture Organization (FAO) and the Office International des Epizooties (OIE: World organisation for animal health). The recommendations and conclusions of these groups vary, for example in the assessment of risk: many EC working groups refer to the likely presence of BSE in sheep, especially in the UK, whereas the OIE persists in considering the risk hypothetical (46).

The recommendations from all groups and expert committees, however, invariably include the statement that 'further research is required to arrive at a rapid diagnostic test capable of differentiating BSE from scrapie in sheep'. This has been the situation for the past five years or so: the existence of a risk is well known, but the tools to assess the magnitude thereof are lacking, the major obstacle being the deficiency of a specific differential diagnostic technique.

Several promising new approaches have been published in recent years. One of these, associated with a surveillance system based on a rapid TSE standard-diagnostic method (and with sound epidemiological precepts), may possibly provide the means for answering the question whether and how many sheep have been affected by BSE.

At the time of writing, BSE in sheep under natural conditions is indeed still a hypothetical issue, but one that contains all the elements of another man-made disaster.

The purpose of this contribution is to list what is known about experimental BSE in sheep, the distribution of infectivity in the

host, some aspects of risk assessment and the most promising methods for differentiating BSE from scrapie in the same host.

## Clinical picture

The gross clinical signs of experimental BSE in sheep (22, 24) were basically indistinguishable from classical scrapie. A striking point, however, was the duration of the clinical course: all sheep challenged experimentally by intracerebral or oral route had an extremely short clinical course, varying between one and five days. No obvious difference related to route of exposure or genotype was observed. Transmission experiments carried out in the Netherlands (66), using oral doses of 5 g, resulted in a clinical period of several weeks.

In the experiments of Foster (22, 24), affected sheep showed mostly rapidly progressing ataxia, resulting in recumbency, and some died overnight. Loss of bodily condition was not mentioned and signs of pruritus were found in only one out of six clinical cases.

In early 2002, an OIE ad hoc group (46) concluded that 'it was at least theoretically possible that BSE cases could have occurred in sheep and goats in the field, primarily in countries where BSE risk has been identified. Such cases, should they exist, are likely to be diagnosed and reported as scrapie.' In view of the relatively short duration of the clinical phase of disease in experimental BSE in sheep, whether such neurological cases with a duration of a few days will be suspected as scrapie when they are observed in the field remains to be seen. The same question applies to serially passaged BSE in sheep: the phenotype may well be different.

The above oral transmission experiments were carried out with 0.5 g of bovine BSE-positive brain material. Other experiments are in progress using even higher doses (5 g) for the oral studies (also see section entitled 'Experimental transmission of bovine spongiform encephalopathy in sheep'). Whether such doses can mimic 'natural' exposure constitutes an additional question.

## Epidemiology

### Risk assessment

Up until present, any country-wide TSE-risk assessment was performed with a view to assessing the situation in cattle, not in sheep. Accurate estimates of the risk of BSE in a certain sheep population are not available. However, as a first approximation, it can probably be accepted that the risk of BSE being present in the small ruminant population of a country is related to the geographical risk of BSE in cattle in that country.

Two extreme situations can be cited to illustrate this: most researchers and probably most policy makers would agree that the risk for small ruminants being infected with BSE would be

the highest in the UK, whereas this risk would be non-existent in New Zealand.

One conclusion of a TSE working group of the EC on this topic was that regarding the risk of BSE in sheep, the bovine BSE risk assessment could not simply be copied to the situation in sheep (16). The group concluded that assessing the risk of BSE in domestic flocks of small ruminants would require the development of a new methodology. This methodology would have to comprise the same elements as those of the geographical BSE risk (GBR) assessment for cattle, i.e. assessing the risk of feed-borne transmission to small ruminant populations, dates and efficacy of feed bans, origin and possible recycling of ruminant-derived MBM, efficacy of rendering processes applied to this material, etc. However, any geographical risk assessment exercise in small ruminants also has to take into account that the BSE agent, once introduced into this population via contaminated feed, is likely to be recycled and amplified via horizontal, vertical and even 'diagonal' routes. Not everything is known yet about the transmission of scrapie, although all the above-mentioned routes seem possible. However, virtually nothing is known about the transmission of BSE in small ruminants. Whether experimental BSE in sheep may be transmitted naturally between sheep like scrapie is a crucial question. Experiments to this end are in progress.

## Different management practices

### Sheep

Any geographical risk assessment performed for BSE in sheep populations would have to take into account large differences in management of various sheep flocks and breeds. A single risk assessment for a whole country therefore does not seem feasible.

Management and feeding practices vary considerably both within the European Union (EU) and among other countries (listed for example in 16, Annex 6). Main differences in feeding are related to differences in the use of the animals, i.e. for meat, wool or dairy purposes.

Sheep kept principally for wool, especially the fine wool breeds, are most often extensively managed on pasture, and not intensely fed. Hence the risk from feed is expected to be smaller for such sheep.

Sheep kept for meat, or for meat and wool, are usually given concentrates at least during late pregnancy and early lactation, often from six weeks before lambing until a month after, depending on the type and quality of pasture or roughage. Concentrates are also fed around mating time ('flushing').

The term 'concentrate' is used in this paper for all compound feeding stuffs, whether farm-mixed or proprietary concentrates. The term is not used when referring to supplementation with

grain alone, particularly as this practice is thought not to constitute a hazard in this respect.

In countries where animals are kept indoors throughout the winter, daily feeding of small amounts of concentrates is not uncommon. In sheep flocks where mating of lambs is common, the animals are often given concentrates from the age of about five months.

Young, still suckling lambs may have access to or start eating small amounts of concentrates when their mothers are being fed. In most housing and feeding systems, lambs have access to feeding troughs, sometimes reserved intentionally for the lambs ('creep-feeding'). As pedigree lambs or replacements are most often only selected between the age of three to five months, many of these animals have thus eaten concentrates very early in life. In countries in Scandinavia for instance, sheep are fed indoors for several weeks after lambing until turnout to pasture and lambs often have access to concentrates.

The main market is for meat production or fat lambs and such lambs are often fed concentrates from an early age. In the UK, as well as in other countries, early fat lambs are slaughtered for the Eastern trade. However, there is also a market for yearlings and older sheep. As regards the production of meat for specific ethnic niche products, sheep of older ages are sometimes preferred.

Milk production in sheep is a less common practice, but some countries have a highly developed sheep milk industry. In such flocks, feeding of concentrates is relatively common from an early age and during most of the year, to boost milk supply. Such populations may be considered to have been exposed to a higher risk.

### Goats

The situation is similar for goats, which are, at least in the present European Community, mainly kept for milk. A small amount of concentrates is often provided to kids from about two weeks of age. The amount is increased gradually as the kids grow. Adult goats are fed protein-enriched feeds, most often containing concentrates, throughout the whole lactation period, which may amount to six months per year. Under such management regimes, goats could have been exposed to a relatively high risk. Should risk assessment be envisaged for small ruminants, goats would require a separate assessment, not only for reasons of different management practices, but also with regard to genetically determined susceptibility, as sheep and goats are far from comparable in this respect.

## Feeding practices

Some figures on concentrate feeding practices in the UK are given in a paper prepared for the SEAC in 1996 (61). As an example, annual consumptions for sheep kept under various conditions are shown in Table I.

**Table I**  
**Annual consumption of concentrate feed in kilograms for**  
**different categories of sheep flocks in 1993 (and 1994)**

Category of sheep	Lowland spring lambing	Early lambing	Upland spring lambing	Hill flocks
Ewes	48 (56)	56 (67)	41 (49)	21 (31)
Lambs	15 (18)	76 (81)	Nil	Nil

Source: Data extracted from *Meat and Livestock Commission Sheep Yearbook, 1994*

No definite published data are available as to how high inclusion rates were for MBM in sheep rations. In general, sheep seem to find MBM less palatable than cattle and inclusion levels in sheep rations may therefore have been less than in those intended for cattle. A report by Det Norske Veritas (DNV), an independent risk management foundation, estimated the use of MBM for sheep rations to be around 1% of the total UK production (12). Another reason for lower inclusion rates would be the lower demand for undegradable dietary protein for sheep rations as compared with rations for dairy cattle for example, balanced for maximum production levels.

### Estimating the magnitude of the bovine spongiform encephalopathy epidemic in sheep

Breeding sheep rations in the UK may have contained 1% to 5% MBM, similarly to grower and finisher rations. Given an uptake of concentrate of approximately 50 kg and an inclusion rate of MBM of 2%, for example, this would mean that sheep could consume approximately one kg of heat-treated MBM in one season. Given the fact that, experimentally, sheep may be infected orally with 0.5 g of fresh brain material, this level of inclusion may have entailed a risk.

Such inclusion levels of MBM would probably not have led to patent infections in slaughter lambs as these are killed at an early age, but could have given rise to index cases in breeding ewes. Another DNV report (13) estimated that sheep were maximally exposed to an infectivity level of a thousand-fold less than the cattle oral lethal dose 50 ( $LD_{50}$ ). This implies that up to 5,100 cases of BSE in sheep could have been caused by the uptake of MBM (13). Reassuringly, the factor is more likely 10- or 100-fold less, implying only 500 or even 50 index cases.

These estimates concern the peak exposure year for animals, just prior to the initial MBM feed ban implemented in the UK in July 1988. Exposure may have continued at a lower level in later years, due to cross-contamination. Index cases are likely to have stopped being 'created' following the total ban on MBM for all farm animal implemented in the UK in August 1996 and in most other EC countries by January 2001.

Recently, further attempts have been made (19, 38) to estimate the theoretical size of an epidemic of BSE in sheep on the assumption that sheep had been exposed via feed. Kao *et al.*

estimated that between 10 and 1,500 sheep may have been infected with BSE at the peak of the epidemic, the extremely wide range of possibilities in this case being mainly due to uncertainties in underlying data (e.g. the rate of maternal or horizontal transmission). Furthermore, the fact that the calculations are theoretical should also be considered.

Another option to estimate the potential size of the BSE epidemic in sheep would be to assess, by strain-typing, the fraction of BSE-like strains among the total TSE occurrences in sheep. This has been tried with two collections of scrapie samples, using classical strain-typing in mice as the discriminating method.

The one study, mentioned previously in this paper, involved almost 3,000 scrapie-positive brains, collected in the 1980s and early 1990s in the framework of a rendering study (64). The samples were later thought to offer an opportunity to search for sheep BSE. However, due to possible contamination or mixing with bovine brain material, the study did not yield useful data on whether BSE was present in sheep.

The other study involved approximately 200 brains and is still ongoing. So far, as reported by the Food Standards Agency on 2 August 2001 (20), no BSE-like isolate was found in this group of carefully collected UK specimens, dating from the late 1990s. The study provided some reassurance that the BSE agent did not occur very frequently among the TSE samples collected from UK sheep. Stating more than that was unfortunately not possible as the samples did not seem to have been representatively collected: up to ten cases had been collected from individual flocks, so a certain amount of clustering would have occurred.

In a SSC opinion on statistically authoritative BSE/TSE surveys (18) the results of the latter study were used as an example. The committee concluded that the upper 95% confidence limit for BSE prevalence in clinical TSEs in sheep was approximately 2.5%, the issue of clustering was, however, not addressed.

Had this initial exposure of small ruminants indeed occurred in a substantial manner, there would have been an increase in the reports of TSE cases in local sheep populations, even assuming a certain degree of under-reporting. Epidemiological analysis of available data on scrapie incidence in the UK did not indicate a rising trend in the disease as a result of the BSE epidemic in the cattle population (30).

An interesting option was also discussed in the same SSC opinion, i.e. whether sheep on farms with cases of bovine BSE would be at higher risk. In the UK, apparently, TSE cases in sheep on farms with a case of bovine BSE did not occur at a significantly higher rate than on those without a case of bovine BSE (F. Courtin, unpublished observation).

In conclusion, for the initial, theoretical index cases of BSE in small ruminants, risk factors are very similar to those used in

risk assessment studies for cattle. Based on the lower level of concentrate feeding to small ruminants in general, the risk of BSE occurring in small ruminants in a certain country is likely to be proportionally related to the risk of BSE in cattle of that same country.

To assess the risk that infectivity be passed on to other animals once introduced in a national system, i.e. the propagation risk, a number of 'sheep-specific' aspects should be taken into account. One major factor to be considered is the transmission efficiency to other sheep (for which proper data is still lacking). Other factors include species-specific measures to eliminate infectivity from the food- and feed-chain, e.g. culling and destruction of suspect cases, the culling policy for the rest of the flock and the efficiency of specified risk materials (SRMs) ban. Some of these measures for small ruminants were implemented much later than those concerning cattle.

## Experimental transmission of bovine spongiform encephalopathy in sheep

The first reported successful experimental transmission of BSE to sheep and goats dates back to 1993 (22). This early study, performed at the Institute for Animal Health in the UK, involved oral challenge of Cheviot sheep with 0.5 g of BSE-infected bovine brain. Transmission only occurred in a proportion of sheep, with the shortest incubation periods being observed in sheep of the genotypes ARQ/ARQ and AHQ/AHQ (polymorphisms at codons 136, 154 and 171 of the prion protein (PrP) gene are indicated here, e.g. ARQ: alanine, arginine, glutamine; AHQ: alanine, histidine, glutamine) (24, 25). Intracerebral challenge of Cheviot sheep of similar genotype resulted in infection of the majority of challenged animals. Sheep carrying at least one ARR (alanine, arginine, arginine) allele did not succumb, irrespective of the route of infection.

More studies on experimental transmission of BSE to sheep have been reported since or are presently ongoing and have been extended to Suffolk sheep (S. Bellworthy, unpublished data). Most experiments are still in progress, but ARQ/ARQ Romneys have already been shown to be readily susceptible to infection, with incubation periods ranging from 550 to 1,130 days. In the study, 5 g of BSE-infected bovine brain was used for the oral challenge. In Romneys with ARQ/ARR and ARR/ARR genotypes, no evidence of clinical disease is observed at 40 months and immunocytochemistry or bioassay show no evidence of infection of the central nervous system (CNS) or peripheral tissues (36).

In the Netherlands, six ARQ/ARQ Texel sheep, infected with 5 g of BSE-infected brain by oral route were also shown to be susceptible (66). Incubation periods were approximately two years. In France, four Lacaune sheep (ARQ/ARQ) were exposed

parenterally (splenic or intraperitoneal routes) and three of these animals were successfully infected (M. Savey, personal communication, 2002).

In addition to Cheviot sheep, Romney, Suffolk, Lacaune and Texel sheep have also been shown to be susceptible. The majority of sheep in which oral transmission has been proved have been of the ARQ/ARQ or AHQ/AHQ genotype. Two other genotypes, one AHQ/ARQ and one VRQ/ARQ (VRQ: valine, arginine, glutamine) sheep, also succumbed to experimental infection with BSE.

None of the experimental challenges performed to date have replicated probable natural infectious doses via feed or by horizontal or maternal transmissions. The 5 g challenge guaranteed an almost 100% score in susceptible genotypes, but exposures to low doses of infectivity must also be studied. A range of experiments are planned or have already commenced, mainly in the UK, using oral exposure levels ranging from 0.0005 g to 5.0 g of BSE-infected bovine brain. In addition, a range of genotypes representing the major alleles found in the UK sheep population has been selected for experimental challenge. The breeds used in the UK include Cheviot, Romney, Suffolk and Poll Dorset, some of which were imported from New Zealand to generate freedom from infection with scrapie. In total, several hundred sheep have been or will be challenged, principally in UK-funded projects, and further developments may therefore shortly be expected in this field.

Studies involving oral challenge of sheep with brain tissue from sheep experimentally infected with BSE (i.e. sub-passage in sheep) are also ongoing. These studies should produce essential information and could shed more light on the stability of BSE in sheep when endemic, as well as on the possibilities of recognising the disease as such.

### Interaction between genotypes and transmissible spongiform encephalopathies

The relationship between sheep genotypes and susceptibility to infection with transmissible spongiform encephalopathies is discussed in more detail in a previous chapter on scrapie (14).

Briefly, the genotype of the sheep, and specifically codons 136, 154 and 171 of the PrP gene, play a part in conferring susceptibility or resistance to infection with scrapie. No single genotype confers universal susceptibility. In other words, susceptibility also appears to depend on the strain of scrapie agent to which a sheep is exposed, the most well-known exception to the rule being the CH1641 strain (21).

Codon 136 of the PrP gene normally encodes for the amino acids valine (V) or alanine (A). Codon 154 encodes for arginine (R) or histidine (H), while codon 171 encodes for arginine (R), glutamine (Q) or more rarely, histidine (H). The three-character coding used above describes which amino acids are coded for

on these three important loci. For example, a sheep with the genotype ARQ/ARR has one allele with A at 136, Q at 154 and R at 171, and another allele with A at 136, R at 154 and R at 171.

At least 15 genotypes have been recognised in sheep breeds as a result of the possible combinations, but in the majority of breeds, the genotype range is far more restricted.

Published data on confirmed cases of scrapie world-wide suggest that sheep that are homozygous for arginine (RR) at codon 171 (genotype ARR/ARR) are not susceptible to infection with scrapie. Preliminary experimental transmission data show that this genotype may also confer resistance to infection with BSE, at least following oral exposure. Results of ongoing experimental challenges in the UK may confirm this. However, at the time of writing, one of a group of 19 New Zealand ARR/ARR sheep was reported to have succumbed to intracerebral inoculation of brain material from a BSE-infected cow after an incubation period of 33.5 months (11). The implications for the genetic breeding programme are not yet clear, but it is assumed that the epidemiological basis of the programme will not be affected as the infection route is so different from natural exposure. The results resemble those of experimental BSE challenge of pigs a decade earlier.

The 'innate' or intrinsic resistance to BSE of the genotype ARR was already alluded to in earlier *in vitro* conversion assays (53), in which the molecular resistance for conversion into the pathological form of the PrP was assessed. Both scrapie and BSE were unable to convert the cellular form of PrP<sup>ARR</sup> beyond a few percent.

Prion protein genotypes conferring resistance to scrapie and BSE may be similar but a noteworthy difference exists, however, with regard to the most susceptible genotype. Whereas, in many breeds, the VRQ genotype appears to be the most susceptible for most scrapie strains and also shows the shortest incubation periods, sheep with the ARQ allele seem to be the most readily infected by the BSE agent (24, 25). Interestingly, *in vitro* conversion of the PrP<sup>ARQ</sup> by the pathological form of the prion protein in BSE (PrP<sup>BSE</sup>) was about three times more efficient than that of the PrP<sup>VRQ</sup> (53). Furthermore, the *in vitro* conversion assay provides an indication of the extent of the species or transmission barrier.

## Distribution and kinetics of infectivity in the host

At the time of writing, only two studies have been published on tissue distribution of infectivity in experimental BSE in sheep, one of these being an endpoint study with animals in their terminal stages (24), while the other study also provides some preclinical data (36). Although the latter experiments are still in progress, ARQ/ARQ Romneys have already been shown to be

readily susceptible to oral exposure with 5 g of BSE material, with incubation periods ranging from 550 to 1,130 days. A range of tissues was found positive by immunohistochemistry or bioassay, namely: in addition to the CNS, large sections of the intestine, the lymphoid tissues associated with it, and other organs such as the liver and pancreas (36). The earliest appearance of disease was at four months after inoculation, in a retropharyngeal lymph node of one out of four animals.

Romneys with ARQ/ARR and ARR/ARR genotypes exposed to the same oral dose showed no evidence of clinical disease at 40 months and no evidence of infection of the CNS or peripheral tissues was observed by immunocytochemistry or bioassay.

The resistance of the ARR/ARR genotype to BSE infection was also confirmed by experiments in France (O. Andreoletti, personal communication). In these experiments, the animals were infected parenterally by intra-splenic or intraperitoneal injection (AFSSA in 46). However, the intracerebral route of exposure apparently overcomes part of the resistance barrier (11).

Although published information may still be limited (36), the pathogenesis and tissue distribution of infectivity in cases of experimental BSE in sheep, appear to differ from those in bovine BSE and resemble more those in sheep scrapie. Similarly to scrapie, the most striking difference is the involvement of the lymphoid system. Information for control measures could therefore also be retrieved from additional scrapie data.

A very recent finding confirms that blood may carry infectivity in the case of sheep BSE (33, 34, 35). This will raise questions about the safety of whole carcasses, but probably also that of milk.

## Possibilities for differentiating between bovine spongiform encephalopathy and scrapie in sheep

### General histopathology

The neuropathology of a TSE in a particular host may be distinctive and, together with other features, provide significant information as to the origin of the disease in that host. In the strictly controlled mouse models of scrapie described in the next section, a method of scoring the intensity of vacuolation in selected brain areas at the late clinical stage of disease provides the 'lesion profile'. This can be considered a characteristic 'fingerprint' of a particular isolate, which, together with an established incubation period, constitutes a major experimental approach for the determination of different strains of TSEs.

Profiling of vacuolar changes directly in the affected host has also been investigated as a possible approach to determine the origin of a TSE. Lesion profiling has been applied to the brains of cattle with BSE to define the vacuolar pattern of the disease (57, 68). The striking uniformity of this profile over the years (M.M. Simmons *et al.*, unpublished data) and the wide geographical distribution of the disease (47) added further support to the notion that the epidemic has been sustained by a single strain of agent.

The situation is entirely different with scrapie. So far, characterisation of the vacuolar pathology of the brains of affected sheep has not enabled strain recognition. In fact, strain characterisation using the mouse model was developed as a direct result of the difficulties presented by the variation observed in the pathological features of experimental scrapie in sheep. This variation alone is sufficient to indicate that differentiating BSE from scrapie in sheep would not be possible by the method of characterising vacuolar changes or other pathological features. However, differences in patterns of PrP accumulation appear to offer a more promising route for the identification of sheep scrapie strains (see section entitled Immunohistochemistry, 'prion protein profiling').

### Classical strain-typing

The most widely recognised method for differentiating strains of TSEs is based on transmission and serial passage of isolates in a panel of inbred mouse lines. This panel consists of a minimum of three mouse lines: RIII, C57BL (both *Sinc<sup>ST</sup>*) (*Sinc*: a mouse gene exerting control over Scrapie incubation period), and VM (*Sinc<sup>PT</sup>*), all bred in the Neuropathogenesis Unit (NPU) of the Institute for Animal Health in Edinburgh. Originally, a C57BL × VM F<sub>1</sub> cross was also used. The NPU can be regarded as having laid the basis for this form of strain-typing. Almost all evidence that multiple strains of TSE agents exist has been provided from these animal models. Similar evidence has been obtained from the passage of transmissible mink encephalopathy (TME) in hamsters. For scrapie agents, strain discrimination is based on the phenotypic characteristics of the disease expressed in mice. These characteristics include incubation period, and pattern of distribution and relative severity of the spongiform changes in the brain (the 'lesion profile'). Such characteristics should remain stable on serial sub-passage, in order to qualify as a 'strain'. A recent overview is given by Bruce (7). One setback is that not all scrapie isolates are readily transmissible to the standard set of mice used (7). While there are various distinct strains of scrapie, so far only a single strain of BSE has been identified, which properties appears to be maintained upon passing through intermediate hosts. Re-isolated from the cat, greater kudu (*Tragelaphus strepsiceros*), nyala (*Tragelaphus angasi*), pigs and also man, the BSE agent showed characteristics in the mouse panel remarkably similar to those of an original BSE isolate from cattle (5, 6). The same applies to the isolate from sheep that had been

experimentally infected with BSE (23): it had the BSE fingerprint in terms of incubation periods and lesion profiles.

The consistency of the biological characteristics of BSE in mice on primary isolation has enabled some confidence in identification of the BSE agent in an isolate by single transmission to mice. For this latter purpose, a primary passage into three mouse lines, namely: RIII, C57BL and VM, has been proposed. This simplification results from the observation that, in most cases, the BSE strain is transmitted to all inoculated mice at first passage, contrary to most scrapie sources. Biotyping of BSE and variant CJD (vCJD) agents has therefore been accepted largely upon primary transmission from the host species, which contrasts with corresponding work on scrapie of sheep, where serial sub-passages in mice are required.

The method of primary isolation in these three mouse lines has also been used for the screening of the two collections of scrapie brains described above. When properly applied, the strain-typing method is rightfully considered the gold standard for discriminating between strains of TSEs, although the technique is laborious, costly and requires extensive training and standardisation.

### Immunohistochemistry, 'prion protein profiling'

Differences in patterns of PrP accumulation in different areas of the brain appear to offer a more promising route for the identification of sheep scrapie strains than the characterisation of vacuolar changes. Different cellular and anatomical types of scrapie-associated prion protein (PrP<sup>Sc</sup>) accumulation are found in the brain of scrapie-affected sheep of different breeds and PrP genotypes. These types of PrP<sup>Sc</sup> accumulation may be scored and arranged into patterns or 'profiles'. Differentiation of at least some scrapie sources seems possible this way. Some authors claim that this method also offers scope for distinguishing between sheep scrapie strains and BSE in sheep (26, 37).

Jeffrey *et al.* (37) have combined their approach using PrP antibodies directed at certain peptides of the protein with PrP profiling and claim to be able to discriminate between sheep scrapie sources and BSE infection of ARQ/ARQ genotype sheep (including discrimination within the lympho-reticular system). This approach has not been applied to sheep BSE of other genotypes and only a limited range of natural sheep scrapie sources have so far been examined using this system of discrimination.

Comparable work was also performed at the Central Institute for Animal Disease Control in Lelystad in the Netherlands (66, 67), where tonsil biopsies of BSE- and scrapie-infected Texel sheep were examined for possible differences. Using a panel of anti-peptide antibodies directed against several epitopes along the full length of the ovine PrP, some interesting observations were made when examining immunohistochemically stained sections of the follicles. With antibodies directed against the 89-111 region (the same region as Jeffrey *et al.*) some consistent

differences in the morphology of PrP<sup>Sc</sup> granules in follicular macrophages of BSE- or scrapie-infected sheep were detected: in BSE-infected sheep, PrP<sup>Sc</sup> accumulation was seen as a single granule within the macrophage cytoplasm whereas in scrapie-infected sheep, numerous multiple PrP<sup>Sc</sup> granules could be observed. With other antibodies, directed against other epitopes, this was not the case. Again, this work was performed with only one genotype and breed of sheep.

A possible explanation for this difference is that BSE- and scrapie-derived PrP<sup>Sc</sup> may differ in degradation in macrophage lysosomes. Scrapie-derived PrP<sup>Sc</sup> would not be digested in the lysosomes, explaining the multiple granules, whereas BSE-derived PrP<sup>Sc</sup> would be. This difference in digestibility would then need to take place at sites beyond the 89-111 region.

The possible difference in digestibility of both BSE- and scrapie-derived PrP<sup>Sc</sup> has also been a starting point for several of the methods described in the following section.

### Molecular or biochemical methods

The PrP is a host glycoprotein that has two sites for N-linked glycosylation and can be found in all three states, the aglycosyl, monoglycosyl and diglycosyl forms. Scrapie-associated PrP comprises an abnormal form of PrP that is deposited in TSE-infected tissues. The PrP<sup>Sc</sup> is operationally differentiated from PrP<sup>C</sup>, the normal form of the protein, by its partial resistance to protease digestion. There has been extensive documentation of differences in PrP<sup>Sc</sup> between TSE isolates, usually depending on the measurement of apparent molecular weights ( $M_r$ ) of the aglycosyl form of PrP<sup>Sc</sup> after proteinase K digestion and determination of the relative amount of the three glycoforms of PrP<sup>Sc</sup> by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE). The two measurements, apparent  $M_r$  (or comparative migration on gels) and the degree of glycosylation do not appear to be correlated. However, several factors affect both. The major influence appears to be the strain or source of TSE agent, but there are also influences from the host which can include PrP genotype, tissue type and location within the brain. This has been narrowed down in some cases to cell type. Since these two types of structural variations are presumed to occur independently, they are discussed separately below.

Although not directly relevant to this review, it is worth noting that the origins of these differences in PrP<sup>Sc</sup> structure are a matter of some controversy. Some suggest that the diversity of sizes of PrP<sup>Sc</sup> after protease digestion is a reflection of differences in conformation that encode genotypic differences between TSE strains. Others suggest that changes in size and glycoform ratio reflect phenotypic differences that are not directly related to the transmission of the 'genetic' information of the agent.

### Differential glycosylation of PrP<sup>Sc</sup>

The relative amounts of the aglycosyl, monoglycosyl and diglycosyl forms of PrP<sup>Sc</sup> are measured densitometrically by immunoblotting. The PrP<sup>Sc</sup> from different isolates varies in the degree to which it is glycosylated (9, 39, 59, 60). The degree of glycosylation is also affected by tissue type (29) and region of brain (58). Different ratios of the three bands are found with different antibodies, presumably because in some cases, access to the epitope is affected by the presence of the carbohydrate moiety. Measurement usually depends on digitising the image directly or via photographic film and then using computer software to estimate the areas of the three peaks assigned to the three glycosylated states of PrP. The use of photographic film can be effective. However, because of the limited dynamic range of roughly two orders of magnitude, overexposure of the film must be avoided. Imaging instrumentation, e.g. using a charge-coupled device camera, is preferable since this technique has a much wider range, although it is much more expensive than film.

### The size of the aglycosyl moiety of PrP<sup>Sc</sup> after protease cleavage

After purification of PrP<sup>Sc</sup> and before or after proteinase K digestion, a population of protease-cleaved PrP molecules that vary in their N-termini can be identified by amino acid sequencing. In an early study of PrP<sup>Sc</sup> from mouse brain infected with the ME7 TSE strain, endogenous proteases had cleaved the N-terminus of PrP<sup>Sc</sup> at a range of sites (31). Proteinase K mostly cleaved the PrP<sup>Sc</sup> from this model in the same region, although the precise sites differed. After protease digestion, cleavage mainly occurred at the residue equivalent to G82 of the human PrP sequence (31). For simplicity, all references herein to amino acid sequence numbers are referred to as the human PrP sequence. In an experimental scrapie model in hamsters, the main cleavage site was located at G90 (45, 51). By contrast, cleavage sites were found to vary for two TME-derived strains in the hamster (4), reflecting differences in migration of the aglycosyl band on SDS-PAGE. The faster migrating source had a major cleavage site at G92 and minor one at Q98 and K101. The N-terminus of the slower migrating source was not clearly identified, but was about ten residues N-terminal of the faster source. Several N-termini were thought to be present in both sources. In a series of samples from human TSEs, PrP<sup>Sc</sup> was cleaved at one of two major sites (G82 and S97), although sometimes up to five of nine cleavage sites were identified. In some cases, both major sites were detected (50). Overall, these data show that the major site of cleavage can vary according to the TSE source, although it should be noted in the human series that sporadic CJD cases had both the M/M, M/V and V/V polymorphisms at PrP codon 129. Other factors that may affect these observations such as differences in amino acid sequence of PrP between species, tissue type, or regional distribution have not been systematically investigated by this most rigorous method. The underlying reason for the existence of size differences is not fully understood but they are presumably related to variations in PrP<sup>Sc</sup> conformation.

However, differences in sequences between species (Fig. 1) may affect favoured sites of cleavage by proteinase K.

Human	80	GWGQPHGG–GWGQGGGTHSQWN	100
Cow		.....G.....–.G...	
Sheep		.....G.....–S.....	
Hamster		.G.....–.G.G.....N...	
Mouse		S.G.....–.....N...	

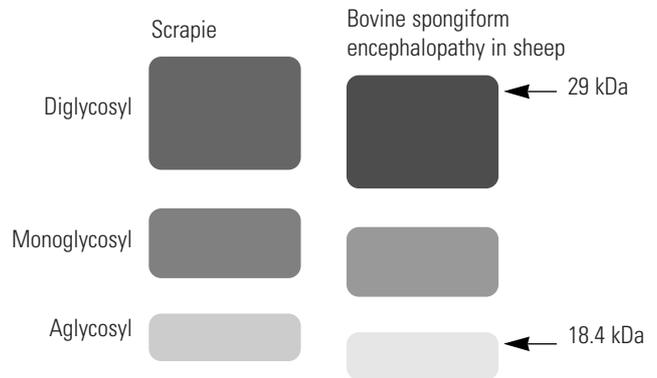
**Fig. 1**  
Differences in sequence with potential effect on cleavage sites  
Courtesy of J.P.M. Langeveld

Differences in cleavage sites result in small differences in the migration of the aglycosyl form of PrP<sup>Sc</sup> on SDS-PAGE, which are usually detected by immunoblotting. Removal of the N-linked carbohydrate by digestion with N-glucosidase F (PNGase) increases the amount of the aglycosyl form available for analysis. Gel analysis does not provide sufficient resolution to detect all the cleavage forms of aglycosyl PrP<sup>Sc</sup>, but different relative amounts of each cleavage form may lead to small differences in the peak migration of PrP<sup>Sc</sup>. What is observed by SDS-PAGE is presumably a reflection of the majority population size, rather than a single PrP species with a unique N-terminus. Although it has been suggested that these small differences of migration may be useful, the difficulty of distinguishing such small variations must be taken into account. These minor differences may be influenced by preparative conditions.

Sometimes two aglycosyl bands can be observed on SDS-PAGE, presumably when both major cleavage forms (at codons 82 and 97) are present in similar amounts. Recently, several peptide or monoclonal antibodies which recognise epitopes between the two major cleavage sites have become available and their use in immunoblotting or histochemistry allows positive or negative determination of the presence of the cleavage forms, even when glycosylated.

**Using size differences of aglycosyl PrP<sup>Sc</sup> to characterise transmissible spongiform encephalopathy sources**

Size differences are found for different TSE sources and have most often been analysed by immunoblotting. They were first analysed in a series of human TSE cases (48), revealing a correlation between clinico-pathological phenotype, PrP genotype and band size. Comparison of a series of human TSE cases showed that vCJD cases had faster migrating bands than other human TSEs and were similar to authentic BSE-derived samples (9, 49). Analysis of sheep TSE cases showed that the aglycosyl band from experimental sheep BSE migrated faster than natural scrapie PrP<sup>Sc</sup> (Fig. 2a). However, one experimental source, CH1641, migrated similarly to BSE (2, 28, 32, 62).



**Fig. 2a**  
The general migration pattern of bands with different degree of glycosylation, for scrapie and bovine spongiform encephalopathy in sheep  
Courtesy of J.P.M. Langeveld

Scrapie-associated PrP from a series of sheep scrapie cases in France were found to have similar migration properties to cattle BSE (1), but were later found to migrate slower than experimental sheep BSE samples (2). Immunohistochemical examination with a panel of anti-PrP antibodies allows discrimination between TSE sources but is also informative about tissue specific, regional and cellular effects. Specifically, antibodies to epitopes between the major cleavage sites should stain PrP<sup>Sc</sup> differentially after cleavage. Indeed these reagents allow analysis to be directed towards particular regions of the brain and cell types within that region (37).

**Conclusions**

Two major potential cleavage points have been identified in hamster and human PrP<sup>Sc</sup> and similar duality probably also exists in other species, although sequence analysis has not been performed. The precise position of the major cleavage sites may be affected by differences between PrP sequences in different species. Other more minor cleavage sites have also been identified. Differences in cleavage are presumably responsible for differences in apparent size observed by SDS-PAGE. Minor differences in size may be difficult to demonstrate routinely. The size of the aglycosyl form of PrP<sup>Sc</sup> after protease digestion can vary according to the TSE source, but can also be affected by host factors. Samples from BSE-derived sources tend to have smaller apparent size than most, but possibly not all, scrapie sources. Small PrP<sup>Sc</sup> fragment size may therefore be of use in identifying candidate BSE cases but since some other TSE sources also have small sized fragments, this cannot be considered a unique marker for BSE.

**Potential of biochemical analyses for differential diagnosis**

**PrP<sup>Sc</sup> in experimental models**

Analysis of mouse passaged TSE strains showed that the degree of glycosylation varies widely (60). A BSE-derived strain (301V) was glycosylated to a similar extent as some sheep scrapie-derived strains and could not be distinguished from them

because of the degree of experimental variance. A scrapie isolate from Germany resembled scrapie strain ME7, which has frequently been isolated from sheep scrapie in the past. In selected strains or isolates, no influence of the mouse lines used was observed with PrP<sup>Sc</sup> profiles, nor were brain region-specific differences apparent (40), although some have found significant differences (58). Although both differences in the degree of glycosylation and migration of protease digested PrP<sup>Sc</sup> have been demonstrated in experimental TSE models, there has been no attempt to test whether these properties can be used for differential diagnosis. However, they do show some of the potential limitations of the approach. Firstly, the degree of glycosylation varies widely between TSE strains and can be further affected by several other factors, including tissue source and region of the brain sampled.

### PrP<sup>Sc</sup> in human transmissible spongiform encephalopathies

Differences in the degree of glycosylation between human samples have been correlated with PrP genotype and clinicopathological presentation (48). Comparison of a series of human cases showed that the degree of glycosylation of vCJD was higher than other human TSE cases in the series and was similar to other BSE-derived sources (9, 49). Polymorphic residue 129 of PrP had a leading role in determining the proteinase degradation site of PrP<sup>Sc</sup>, while mutant residues 102 or 200 only influence the glycosylation pattern in one study (8). In another study, differences in PrP<sup>Sc</sup> properties were associated with different patterns of PrP deposition and severity of spongiform changes (52). Thus, although some correlation between various features of human TSEs and PrP<sup>Sc</sup> have been sought and found, it is not yet clear whether any unique associations exist between degree of glycosylation and fragment size of PrP<sup>Sc</sup>.

### PrP<sup>Sc</sup> in ruminants

Possessing a rapid method for the differential diagnosis of BSE and natural scrapie in sheep would be highly desirable. The measurement of the degree of glycosylation and apparent size of protease digested PrP<sup>Sc</sup> provides the best option to this end and accordingly, has given rise to several studies summarised below.

In an initial study, all natural sheep scrapie samples exhibited a larger PrP<sup>Sc</sup> fragment size, as did the experimental source SSBP/1. Another experimental source, CH1641, exhibited a smaller fragment size, similarly to experimental sheep BSE. Analysis of the glycoform ratios was not formally presented, although the experimental sheep BSE source appeared to be more highly glycosylated than the other samples analysed (28). A separate analysis, which included many of the same samples, also showed that both CH1641 and experimental sheep BSE exhibited the faster migrating aglycosyl band. Some natural scrapie samples exhibited even faster migration than the CH1641 and BSE samples, an observation not reported by other authors so far. Again, formal glycoform ratio analysis was

not presented (32). Figure 2a shows the general migration picture as found by most authors.

Analysis of a series of natural scrapie cases from France showed little difference, either in the degree of glycosylation or in migration properties, to those of cattle BSE or other BSE-derived sources (1). However, it was subsequently shown that the aglycosyl form from experimental sheep BSE migrates similarly to the fragment from CH1641, but more rapidly than that from bovine BSE and from natural scrapie cases in France (2). In another study, different brain regions from a given sheep displayed a similar glycoform ratio of PrP<sup>Sc</sup>, whereas the apparent molecular sizes of the unglycosylated and diglycosylated forms of the protein differed between brain and lymphoid tissues. No notable differences in the glycoform ratio of PrP<sup>Sc</sup> were found in brains from animals with natural scrapie of different PrP genotypes or clinical status. The PrP<sup>Sc</sup> glycoform ratio was also similar to that found in the brains of four cattle with BSE (42). However, it should be noted that the antibody used (P4) was raised against a peptide sequence (residues huPrP82-104, or ovinePrP85-107) which covers the region of PrP<sup>Sc</sup> that is differentially cleaved. Therefore, possibly only a subset of PrP<sup>Sc</sup> molecules was recognised in these assays. The PrP<sup>Sc</sup> protein in natural sheep scrapie samples from Ireland had lower glycoform ratios than PrP<sup>Sc</sup> from cattle BSE. Similar glycoprofiles were found when analysing scrapie PrP<sup>Sc</sup> from six different CNS regions. No size differences were detected, but again the P4 antibody was used (63).

In the most detailed study to date, a panel of ruminant brain tissues were studied using two monoclonal antibodies (mAbs) (62). The resultant PrP<sup>Sc</sup> glycoforms allowed three distinctions to be made between natural scrapie cases, cattle BSE and experimental sheep BSE. The aglycosyl band from natural scrapie migrated slower than that from experimental sheep BSE. The band from cattle BSE migrated slightly slower than that from sheep BSE. Glycoform ratios distinguished between experimental BSE in sheep and natural sheep scrapie, but not between cattle BSE and natural scrapie. By contrast, CH1641 gave apparent molecular weights similar to, but not identical to, BSE and a glycoform ratio similar to ovine scrapie cases. The SSBP/1 scrapie pool gave apparent molecular weights similar to natural scrapie cases, but the glycoform ratio was lower than that in all the other samples. When mAb P4 was substituting for mAb 6H4, only the natural scrapie samples and SSBP/1 gave strong signals (Fig. 2b). Bovine spongiform encephalopathy in sheep and the CH1641 strain gave weak reactions and PrP<sup>Sc</sup> from BSE-infected cattle could not be detected at all (62).

### Co-infection and resolution of mixtures

The feasibility of resolving a mixture of two PrP<sup>Sc</sup> structural patterns has been tested should co-infection with natural scrapie and BSE occur. Although distinct doublets of the aglycosyl band can be resolved in the same human samples, this was not possible either with artificial mixtures or

PrP antibody	Scrapie	Bovine spongiform encephalopathy
Most*	Yes	Yes
P4	Yes	No

PrP: prion protein

\* Most antibodies with an affinity for ovine prion protein, either monoclonal or polyclonal, can be used for detection of both scrapie and experimental bovine spongiform encephalopathy in sheep (2, 28, 32, 35, 62, 66)

**Figure 2b**  
**Different staining properties of some antibodies (P4 versus most others)**

Courtesy of J.P.M. Langeveld

co-infected mouse brains of mouse passaged BSE and natural scrapie (3). Differences in PrP sequence between species may differentially affect cleavage sites although the authors did not consider this explanation.

### Interspecies transmission

The migration properties of aglycosyl PrP were maintained on transmission of four human TSE sources to mice containing a hybrid mouse-human transgene (65). There has been little study of the same TSE source in different species, with the exception of BSE, which tends to maintain the faster migration form of the aglycosyl band and to be heavily diglycosylated. However, small differences have been found between cattle and experimental sheep BSE. All PrP<sup>Sc</sup> from sheep BSE studied so far has been from primary transmissions from cattle BSE. Subsequent sheep-to-sheep passage may possibly lead to further changes in PrP<sup>Sc</sup> properties.

### Conclusion

The measurement of the degree of glycosylation and the apparent size of protease digested PrP<sup>Sc</sup> has been proposed as a strain-typing tool. In particular, the technique may be useful for differential diagnosis of BSE and scrapie in sheep. However, the use of the method is only valid if certain caveats are observed. Firstly, the differential power of the two measurements is restricted. Differences in apparent size are less than  $M_r = 2000$ , a value close to the limits of resolution of SDS-PAGE. Secondly, the degree of glycosylation can vary over a wide range. The ability of the system to distinguish between two isolates with similar degrees of glycosylation is limited by the variability inherent in the measurements.

In practice, BSE-derived samples show characteristic properties with high degrees of glycosylation and small apparent size of the aglycosyl band. This differs from many TSE isolates, particularly from sheep. However, in some cases, similar migration properties to those of BSE samples have been

observed, notably in the experimentally transmitted sheep scrapie source CH1641.

Overall, analysis of the properties of PrP<sup>Sc</sup>, sometimes referred to as glycotyping, is a useful tool for screening for candidate cases of BSE in the small ruminant population. However, there are several caveats that must be considered, as follows:

- the method must be well characterised with appropriate controls from BSE- and scrapie-infected sheep. The antibodies used must be selected with care. Ones that differentially bind to slow-migrating but not to fast-migrating forms or aglycosyl PrP<sup>Sc</sup> may well be of value when used in combination with an antibody that binds to all forms of the protein
- the system has only limited discriminating power
- some scrapie sources may have similar properties to BSE, although none have yet been identified and BSE in sheep may change properties on subsequent passage
- analysis may be compromised if samples are from different tissues or brain areas.

If candidate BSE cases in sheep are identified from structural differences in PrP<sup>Sc</sup>, directly or through differential immunochemical properties, it will be necessary to consider that these properties may not be unique to BSE. Hence, further neuropathological investigation, or ideally, passage into mice for biological strain-typing will be required to test whether other phenotypic properties of the suspect case match those of BSE or not.

## Risk assessment and management

### Risk for humans, species barrier

Recent publications (19, 38) have attempted to estimate the theoretical size of an epidemic of BSE in sheep on the assumption that sheep had been exposed via feed. Due to uncertainties in underlying data, the range of possibilities is wide and still needs to take into account the possibility of horizontal spread from sheep-to-sheep. If horizontal transmission does not occur, then the current UK sheep population is estimated to contain none or, at most, tens of animals infected with BSE. With significant horizontal transmission, the number would be only slightly higher but could still be rising (38). The same uncertainties apply to attempts at predicting the size of the human epidemic as a result of the exposure from ovine BSE. Ferguson *et al.* (19) estimate that BSE from sheep could easily double the size of the human epidemic, increasing the upper limit to 150,000 human cases instead of 50,000 from bovine BSE alone. Some consolation can be derived from lower limit, which is only 50.

Little can also be said about differences regarding the species barrier, namely: whether a difference exists between transmissibility of bovine BSE to humans and ovine BSE to humans. In other words, would the zoonotic risk be comparable or different? Molecular studies, addressing part of the species barrier, indicated that there is little difference in conversion efficacy between, on the one hand, ovine scrapie PrP<sup>Sc</sup> and human PrP<sup>C</sup> and on the other hand, that of bovine BSE PrP<sup>Sc</sup> and human PrP<sup>C</sup> (53).

### Control measures including consumer protection

Initially, all control measures against the spread of BSE only concerned cattle, i.e. the export bans on live cattle, the initial ruminant-to-ruminant feed ban, the specified bovine offal (SBO) or SRM bans. During that time, exports of live sheep from the UK continued. Any initial risk originating from sheep in the UK could thus have been disseminated to other countries.

Tissue infectivity distribution complicates the applicability of SRM measures for consumer protection. This is mainly due to the early involvement of lymphoid tissues in sheep TSEs: clinical signs can easily take more than a year to appear (27, 54), which is, for the most susceptible genotypes, less than 50% of the incubation period (55). Associated with the possibility of infectivity present in blood (33, 34, 35), SRM for sheep should clearly comprise more than just central nervous tissue.

The first SRM ban for sheep in the UK consisted of banning most parts of the head only (43). A later version of the ban, implemented in the UK in January 1998 (44), included the skulls, tonsils and spinal cords of animals over twelve months, as well as the spleens of all slaughter animals. This measure was more in line with the ill-fated EC Commission Decision 97/534 (15).

Future risk reduction strategies will also have to include a combination of enhanced tissue- and age-based restrictions in the use of animals slaughtered for consumption.

The entire intestine, the lymphoid tissues associated with it and other organs such as the liver and pancreas, seem likely candidates for inclusion in the enhanced tissue restriction measures (36). The finding that blood may carry infectivity in the case of sheep BSE (33, 34, 35), complicates this issue only further, probably to the extent that unless freedom from TSE can be guaranteed, the whole carcass will be considered suspect.

On age-delineation, an EC ad hoc group has tried to establish a diagram where tissue-infectivity titre and age were combined, with the intent to pinpoint the threshold between safe and contaminated categories (Table I in 17). Most of the input data, however, originate from classical scrapie work, principally that

of Hadlow in the early 1980s, and later immunohistochemistry work. These data may therefore not be fully applicable to the present issue. Inputs from experimental work with BSE in sheep as described above have basically not changed this approach so far.

The category between six and twelve months remains problematic: below six months, all tissues of slaughter lambs are generally considered safe (many experimental infections have been carried out with relatively high doses and using the most susceptible genotype, both resulting in a shortening of the incubation period; such genotypes and doses may be rare in real life). However, above twelve months little guarantee can be given and between six and twelve months, a grey zone exists, which only genotyping could resolve, based on a genotype-dependent infectivity appearance. Unfortunately the cost of such testing prevents routine application in slaughter animals. A more suitable approach would be one of the rapid PrP<sup>Sc</sup> detection methods.

In a recent paper (19), an estimation of the effect in risk reduction of various measures was given, as follows:

- removal of all the above-listed SRM except the lymph nodes, would reduce risk of exposure for the consumer by some 60%
- in the same study, this equalled the effect of banning human consumption of all sheep over twelve months of age
- reducing the cut-off from twelve months to six months for allowing slaughter animals to be used for consumption reduces the risks of exposure by a further one-third (of the estimated risk).

Other meaningful results could be obtained from breeding for resistance programmes, which have been initiated in the Netherlands, France and the UK in recent years. In fully resistant animals (homozygous arginine [R] on codon 171) and under natural conditions, neither scrapie nor infectivity was found in Europe. The one notorious exception concerned a case reported from Japan, but which was never confirmed independently.

In addition, heterozygous resistant animals (Q/R 171) could contribute to reduction of exposure, since the appearance of infectivity or PrP<sup>Sc</sup> in the CNS occurs later than in other susceptible genotypes and the degree of lymphoid tissue infection may be less.

### Safety of milk and milk products

Even as late as 2001, the SSC did not issue more than the earlier statement that 'No infectivity has been detected in milk of sheep with scrapie or BSE, but as far as BSE is concerned, the experimental data are very limited' (17). For milk and milk products, there seems to be sufficient reason to be slightly more concerned in the case of BSE in sheep than with cattle. This is, firstly, because of the involvement of the lympho-reticular

system with TSEs, including BSE, in sheep, and secondly, because of the recent detection of BSE and scrapie infectivity in blood. Colostrum could be a particular risk. Further research with regard to BSE and milk safety is ongoing.

## Discussion and conclusions

To date, BSE has not been diagnosed in any national sheep population under natural conditions. However, the possibility that BSE could be present in small ruminants has, over the past few years, been on many agendas of expert committees and is receiving considerable attention from the research community.

Contrary to BSE in cattle, there has not yet been a concrete geographical risk assessment exercise for BSE in small ruminants, probably because sufficient detailed data enabling a specific assessment are lacking. Another simple reason could be that policy-making bodies are too occupied with managing BSE in cattle. For the sake of convenience, it can be accepted as a first approximation that the risk of BSE being present in the small ruminant population of a country is related to the geographical risk of BSE in cattle in that country. However, a more refined assessment would be more tedious and would have to take all sorts of different management and feeding practices into account.

So far, control measures for animal health have concentrated on sharing feed bans and other measures with the large ruminant population, whereas control measures for human health have been accessed on risk reduction strategies, for example, by removing SRM from the food chain. Only in recent years, new control strategies have been introduced, based on genotyping breeding stock: selective breeding for resistance against all TSEs should reduce the possibility of BSE occurring in small ruminants.

As also suggested by an ad hoc group of the OIE (46), the various techniques presently being developed for discriminating between sheep scrapie and BSE in sheep could be used for the initial screening of positive TSE samples from sheep, given the caveats discussed above. Despite major limitations, this initial screening is important because the magnitude of such an exercise should not be underestimated, as shown by an example in one of the SSC opinions (18). To exclude, with 95% confidence, a BSE prevalence of 1 in 200 TSE-positive sheep in the population, strain-typing needs to be carried out in at least 600 TSE-positive sheep, without finding any BSE positive animal. For the 99% confidence interval, this number rises to 920. Classical strain-typing using the standard mouse panel cannot process this sort of numbers and the other more rapid methods described above should be put to use.

However, because none of these other tests have as yet been adequately evaluated, and consequently not validated, for the

purpose of discriminating between BSE and scrapie in sheep, their initial use should be for identifying isolates that appear different from the norm. Such isolates would then need to be inoculated into the classical mice panel for proper strain-typing. Only when sufficient data from lesion profiling support the correlation with the results of molecular methods will it be possible to rely solely on the latter to search for BSE in sheep.

Various alarming rumours that the finding of BSE in sheep was imminent have had a tremendous impact on the livestock industry. Although so far none of the rumours have been substantiated, they do, however, provide a serious warning: one day a BSE-like strain is indeed likely to be found in sheep. As shown above, several groups are presently working on biochemical and other differential strain-typing methods, some of which appear close to their goal. Only one isolate passaged through the established panel of mouse lines with a resulting lesion profile and incubation time pattern indistinguishable from that of BSE would be sufficient to cause much concern.

To avoid hasty decisions following such a finding, the position of animal and public health authorities should be prepared in advance, especially because the question that will be raised at that time will not be an easy one to deal with, namely, 'when a BSE-like strain is found in sheep, have we then:

- a) found evidence of the transition of bovine BSE into yet another host, or
- b) have we finally found supporting evidence for the hypothesis of the ovine origin of BSE?'

Both possibilities are equally plausible but have very different implications for public health.

If the latter were the case, there would hardly be a need for further restrictions or other public health measures with regard to ovine materials in the food chain: did not most expert panels conclude that ovine scrapie in general should not be considered a risk for public health? If, however, BSE is proved to have affected sheep, calls for further public health measures would definitely be heard, probably with similar effects on mutton consumption and prices as the UK already witnessed recently following the first report of experimental infection of sheep with BSE.

Hopefully, a balanced answer will be found by the various research bodies involved on how to consider a first, probably singular BSE-like case in sheep from the field, before the problem actually arises. In the UK, the BSE Contingency Plan in sheep (10) represents an initial answer. Furthermore, a few more years respite can be hoped for, firstly to establish a largely TSE-resistant sheep population and secondly, to gain more insight into the expected size of the human vCJD epidemic and thus, the real risk of BSE for humans.

## Acknowledgements

As mentioned on the first page of this contribution, the authors have made ample use of the sometimes very thorough work performed by various expert committees and working groups. They would therefore like to thank their colleagues on these

groups for their collective efforts without which this article would not have been written. Jan Langeveld is thanked for his critical comments and for providing the figures.

## L'encéphalopathie spongiforme bovine chez les ovins ?

B.E.C. Schreuder & R.A. Somerville

### Résumé

Au moment de la rédaction de ces lignes, l'encéphalopathie spongiforme bovine (ESB) n'a toujours pas été diagnostiquée chez les ovins en conditions naturelles, et ne soulève donc qu'un problème hypothétique. Toutefois, les rumeurs d'une découverte éventuelle d'un isolat apparenté à l'ESB chez le mouton ont suscité un vif émoi, que ce soit dans la filière ovine, dans l'opinion publique ou parmi les instances gouvernementales et réglementaires. Les auteurs expliquent les difficultés de mise en œuvre d'une évaluation du risque et des mesures préventives adéquates en l'absence de cas confirmés.

Ils tentent de dresser l'inventaire de nos connaissances sur la transmission expérimentale de l'ESB chez les ovins, la distribution de l'infectivité chez l'hôte, certains aspects de l'analyse et de la gestion du risque, ainsi que sur les méthodes les plus prometteuses de différenciation de l'ESB et de la tremblante chez cet hôte. En ce qui concerne cette différenciation, de nouvelles méthodes en cours d'élaboration suscitent beaucoup d'espoir, dans la mesure où elles semblent adaptées à un premier dépistage des isolats d'une encéphalopathie subaiguë spongiforme transmissible. Toutefois, à défaut de validation adéquate, l'utilisation du typage « classique » de souches sur souris reste d'actualité.

### Mots-clés

Clivage – Différenciation – Encéphalopathie spongiforme bovine – Évaluation du risque – Glycosylation – Ovin – Revue – Transmission expérimentale – Tremblante – Typage de souches.



## ¿Encefalopatía espongiforme bovina en ovinos?

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### Resumen

En el momento de redactar este artículo no se ha descrito ningún caso de encefalopatía espongiforme bovina (EEB) en la oveja en condiciones naturales, por lo que esa eventualidad no pasa por ahora de simple hipótesis. Sin embargo, han cundido rumores sobre la detección en ovinos de muestras afines a las de la EEB, lo que ha generado gran inquietud en la opinión pública, el sector de producción ovina y los organismos gubernamentales y normativos. Los autores describen las dificultades de llevar a cabo una adecuada evaluación de los riesgos y adoptar las oportunas medidas preventivas en ausencia de algún caso confirmado.

Los autores tratan de pasar revista a todo lo que hasta ahora se sabe acerca de la transmisión experimental de la EEB a ovinos, la distribución de la infectividad en el huésped, determinados aspectos de la evaluación y gestión de riesgos y los métodos más prometedores para distinguir entre EEB y prurigo lumbar en un mismo huésped. Por lo que respecta a esta última cuestión, se están elaborando nuevas y promisorias técnicas que parecen adecuadas para los primeros análisis de muestras sospechosas de alguna forma de encefalopatía espongiforme transmisible. Sin embargo, mientras tales pruebas estén pendientes de la oportuna validación, seguirá resultando indicado el uso de la 'clásica' tipificación de cepas en una batería de ratones.

#### Palabras clave

Diferenciación – Encefalopatía espongiforme bovina – Evaluación de riesgos – Glucosilación – Ovino – Prurigo lumbar – Repaso – Segmentación – Tipificación de cepas – Transmisión experimental.



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