Scrapie and experimental BSE in sheep

Nora Hunter

Neuropathogenesis Unit, Institute for Animal Health, Edinburgh, UK

Scrapie is a natural disease of sheep, but it can also be successfully transmitted between sheep by experimental inoculation. Although BSE is primarily a disease of cattle, it has also infected humans (causing vCJD) and, in addition, can be transmitted orally to sheep bringing concerns that BSE might naturally have infected the UK sheep population. Because of this, scrapie and BSE are being compared and studied in detail in sheep. PrP genotype controls sheep susceptibility and resistance to scrapie and to BSE, and deposition of the disease-associated PrPSc, used as a marker of infection, has the potential to act as a means of identifying BSE-infected animals and describing different pathogenesis mechanisms. Sheep orally dosed with BSE show signs of infection in their blood and this model is of major importance in the study of the safety of blood products for use with human beings.

The prion diseases which have received the most publicity are bovine spongiform encephalopathy (BSE) in cattle and variant Creutzfeldt-Jacob disease (vCJD) in human beings. Both of these diseases are arguably ‘unnatural’ as they are the result of contamination of the relevant food supply with the BSE infectious agent and do not apparently maintain themselves in the population by spreading from one individual to another. In contrast, the prion disease found in sheep (scrapie) does spread from one sheep to another in an infected flock. Scrapie in sheep can be regarded as simply a health and welfare problem for the animals, but it is also a good model for the human prion diseases as sheep, like humans (but unlike mice), have a very varied genetic background and share much of the same variation in disease pathogenesis. This chapter, therefore, reviews what is known about scrapie in sheep in both natural and experimental models and will also address the question of experimental BSE in sheep and how this model is being used to research the issue of safety of blood transfusions. The term ‘TSEs’ (transmissible spongiform encephalopathies) is used throughout as an alternative to ‘prion disease’.

Clinical signs

Descriptions of scrapie have been made for over 250 years involving sheep from various parts of Europe including England and Germany.
Despite being now a notifiable animal disease in EU countries, the exact number of cases occurring each year is unknown: one recent anonymous postal survey estimated the rate of under-reporting of cases in Britain as 87%\(^2\). Some countries are regarded as free of the disease, notably Australia and New Zealand, which have stringent procedures controlling the import of sheep, including many years of quarantine, in order to exclude scrapie.

Clinical signs of natural scrapie in sheep\(^3\) can last from 2 weeks to 6 months and often begin with unusual social behaviour and extreme nervous reactions to stimuli such as human contact. The general condition of the affected animal deteriorates, sometimes accompanied by a change in the fleece colour and often it is this latter feature that is first noticed by the farmer or shepherd. Ataxia is common, and pruritis can result from the animal scratching an apparently intense itch against fence posts or by biting the affected area\(^1\), for example around the base of the tail and occasionally the whole of the side of the body can be denuded of wool. In the final stages of the disease, although the appetite may appear normal, the animals lose the ability to feed themselves and the condition degenerates. Scrapie does not seem to alter reproductive ability until muscle wasting interferes with the ability to move. Lambs can, therefore, be born successfully to mothers in the clinical phase of the disease and rams remain fertile and active even when affected by ataxic signs.

Sheep can be experimentally infected with scrapie by using homogenised brain from affected animals as inoculum and administering by several different routes including intracerebral, subcutaneous, oral and intravenous. The clinical signs are more predictable when a single source of infection is used compared with natural scrapie which has a range of possible outcomes. As an example, with a source of scrapie known as SSBP/1 and in an experimental flock (the NPU Cheviot sheep), clinical signs are of short duration, usually only 2–3 weeks and the predominant sign is lack of co-ordination of gait, sometimes recumbency but with little accompanying pruritis\(^4\). Other experimental sources of infection can produce pruritis in NPU Cheviots, however, so this is likely to be a result of scrapie strain variation rather than a breed characteristic\(^5\). It is not known how many different strains of natural scrapie exist although several studies are underway to try to find out using transmission of the disease to a panel of mouse lines and examining features such as incubation period, brain pathology\(^6\) and biochemistry.

BSE can also be transmitted to sheep by inoculation with affected bovine brain homogenate. Clinical signs vary in the different breeds of sheep used by different researchers. In one reported study in NPU Cheviots, after a long incubation period, the animals were affected by a relatively acute illness of short duration, less than 1 week and sometimes
only a day or so. In this case, the main sign was ataxia with little pruritis. In another study carried out in France with indigenous sheep breeds, there was intense pruritis leading to loss of fleece, and ataxia$^7$ with degenerating condition until death after a clinical course of around 3 months.

**Modes of transmission of TSEs in sheep**

It is not known how scrapie spreads between sheep or between sheep flocks. Infection is thought likely to travel between animals (horizontal transmission) via the oral route gaining entry to the body through the gut-associated lymphoid tissues in Peyer’s patches in the alimentary tract. As it is known that placental tissue can harbour infection$^8,9$, and that sheep will consume discarded placentae, this potential candidate is of great interest particularly as the time of lambing is one of high risk of infection for sheep. However, one of the unproven dogmas about scrapie is that maternal transmission of infection takes place between mother and lamb (vertical transmission)$^{10,11}$. Embryo transfer experiments have been carried out to try to separate in utero exposure from peri-natal exposure, but these studies are difficult to control and conclusions uncertain$^{12,13}$. It does seem that in a scrapie infected ewe, the embryo, at least in early stages of gestation, is not itself infected. At which point thereafter that exposure to infection occurs remains the subject of intense investigation: during the birth process, suckling of colostrum and then milk, physical exposure to infected placental tissue or to a contaminated environment or husbandry instruments/personnel. It may be that there are many sources of infection in the environment of a scrapie-affected flock.

Experimental BSE has also been used in maternal transmission studies, allowing BSE infected ewes to lamb and observing the offspring; however, to date in this study, no transmission of BSE has been observed either in goats or in sheep (Foster & Hunter, unpublished)$^{14}$.

**Genetics**

Whether or not a sheep develops clinical signs of TSE following exposure, either naturally in an affected flock or by experimental injection, depends absolutely on genetics. The association of scrapie with certain blood lines of sheep is so strong that at one time the disease was believed to be a simple genetic disease and that animals with a particular mutation would become sick without any need for an infectious agent (see Parry$^1$). However, it has now been clearly
demonstrated that, although susceptibility to scrapie is genetically programmed, the animal requires to be exposed to an infectious agent in order to become sick\textsuperscript{15,16}.

The gene responsible for control of susceptibility and resistance to TSEs is the PrP gene and, in sheep, the most important amino acids are at numbers 136, 154 and 171 (Table 1). At codon 136, the amino acid specified can be either valine (V) or alanine (A); at codon 154, it is arginine (R) or histidine (H); and, at codon 171, arginine (R), glutamine (Q) or histidine (H). As sheep are diploid there are two chromosomes bearing an allele of the PrP gene and the genotype is usually given with each codon in turn for each allele in turn, \textit{i.e.} one allele could be V\textsubscript{136}R\textsubscript{154}Q\textsubscript{171} – more simply written as VRQ – and a genotype could be VRQ/ARR. Although there are 12 possible combinations of the different amino acids, only 5 alleles are predominantly seen in sheep in many studies from around the world\textsuperscript{17}. Other amino acids in the sheep PrP gene are known to vary but so far the ‘three codon’ genotype gives the strongest linkage with incidence of disease\textsuperscript{18}.

\begin{table}
\centering
\textbf{Table 1} PrP protein polymorphisms and the 5 commonly found PrP gene alleles in sheep
\begin{tabular}{ccc}
\hline
Codon number & Amino acid & Allele \\
\hline
136 & Valine (V) & VRQ \\
 & Alanine (A) & ARQ \\
 & & ARR \\
 & & AHQ \\
 & & ARH \\
154 & Arginine (R) & VRQ \\
 & & ARQ \\
 & & ARR \\
 & Histidine (H) & AHQ \\
171 & Glutamine (Q) & VRQ \\
 & & ARQ \\
 & & AHQ \\
 & Arginine (R) & ARR \\
 & Histidine (H) & ARH \\
\hline
\end{tabular}
\end{table}

\textbf{Experimental scrapie and BSE}

The link between PrP genotype and TSE susceptibility was first noticed in sheep which were part of an experimental study flock – the NPU Cheviot flock\textsuperscript{19}. Since 1960, these animals had been routinely challenged with a source of scrapie known as SSBP/1 (Scrapie Sheep Brain Pool number 1) in order to study, by classical genetic breeding experiments,
the well-established incubation period differences in family lines of the sheep. With the advent of molecular genetics and the discovery of the PrP gene in mice, it was soon found that in sheep, the PrP genotype was linked to length of incubation period and also to resistance to disease\textsuperscript{20}. NPU Cheviots which were homozygous with a valine at codon 136 (VRQ/VRQ) had incubation periods of around 160 days, animals which were heterozygous (VRQ/ARQ or VRQ/ARR) had incubation periods of 260 and 360 days, respectively, and animals which were homozygous for alanine at codon 136 (ARQ/ARQ, ARQ/ARR, ARR/ARR, etc) were resistant to the dose of infection given to them\textsuperscript{16,21}. With another source of scrapie, CH1641, the genetic linkage was different. This time, the most susceptible sheep with shortest incubation period were of ARQ/ARQ genotype, ARQ/ARR sheep had longer incubation periods and ARR/ARR animals were apparently resistant\textsuperscript{22}. When BSE was diagnosed in cattle and it was thought likely that the source of the infection was rendered down sheep brain carcasses, BSE began to be used in sheep studies. It was rapidly established that BSE genetics in sheep were similar to CH1641 scrapie, in that ARQ/ARQ sheep had shortest incubation periods by intracerebral and oral routes of infection\textsuperscript{5,22}. However, CH1641 scrapie differs from BSE in other crucial respects and is not BSE related.

**Natural scrapie**

The laboratory experiments were useful in that controlled studies could then, for the first time, be carried out on sheep whose susceptibility to infection was completely predictable. However, it was also important to establish that the genetics revealed by laboratory studies were relevant to the real situation out in the field for normal sheep. It was rapidly established that the basic genetic rules given above were also shown by natural scrapie. VRQ/VRQ genotype sheep were most at risk of developing scrapie disease\textsuperscript{23}. However, not all breeds of sheep actually have the VRQ PrP gene allele. Breeds encoding VRQ are those such as Cheviots and Swaledales, those without VRQ include Suffolks, in which breed the ARQ/ARQ genotype is highest risk although not every animal of this genotype becomes sick in an affected flock\textsuperscript{24,25}. (These differences in susceptibility are likely to be related to sheep breed differences, however, and little is know about the types of strains of scrapie which naturally infect sheep.)

The VRQ/VRQ genotype is rare, even in VRQ-encoding breeds and, as VRQ/VRQ animals in the UK almost always develop scrapie, it seemed that this genotype was the best candidate if scrapie was really a simple genetic disease – an old hypothesis. However, as it was shown
that it was easy to find VRQ/VRQ sheep (and indeed all of the other susceptible genotypes) in countries known to be free of scrapie\textsuperscript{15,16,26}, the genetic disease hypothesis has now been ruled out. Susceptible genotype and an infectious agent are both required for scrapie to develop and it is, therefore, possible to protect highly susceptible sheep if stringent controls are used to prevent infection\textsuperscript{4}.

There are many breeds of sheep and the PrP genetics with regard to natural infection has become very complex ranging from the simple Suffolk sheep with essentially three genotypes (ARQ/ARQ, ARQ/ARR and ARR/ARR) to Texels which have 15 different genotypes, including VRQ/VRQ\textsuperscript{27}. Nevertheless, a programme of breeding for resistance to TSE infection is possible using this information and, in the UK, an ambitious programme (the National Scrapie Plan or NSP) has recently started to breed UK sheep for higher frequencies of the apparently resistant ARR/ARR genotype (Table 2)\textsuperscript{28}. Because of the importance of this genotype, strong challenges have been mounted to prove its total resistance. Recently, there has been a report that, following injection straight into the brain, even ARR/ARR animals can succumb to infection – in this case using cattle BSE as inoculum (Houston \textit{et al}, Nature 2003, in press). However, the intracerebral route is a vigorous challenge and not exactly a natural route of infection; it is still believed

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Risk of scrapie</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARR/ARR</td>
<td>Most resistant to scrapie</td>
</tr>
<tr>
<td>ARR/ARQ</td>
<td>Resistant to scrapie but offspring may be susceptible depending on genotype of other parent</td>
</tr>
<tr>
<td>ARR/ARH</td>
<td></td>
</tr>
<tr>
<td>ARR/AHQ</td>
<td></td>
</tr>
<tr>
<td>AHQ/AHQ</td>
<td>Higher risk of scrapie in these sheep and in offspring</td>
</tr>
<tr>
<td>ARH/ARH</td>
<td></td>
</tr>
<tr>
<td>ARQ/ARH</td>
<td></td>
</tr>
<tr>
<td>AHQ/ARH</td>
<td></td>
</tr>
<tr>
<td>ARQ/AHQ</td>
<td></td>
</tr>
<tr>
<td>ARQ/ARQ</td>
<td></td>
</tr>
<tr>
<td>ARR/VRQ</td>
<td>Susceptible to scrapie but could be used as a breeding source of the ARR allele associated with resistance</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td>Sheep of highest susceptibility to scrapie in self and offspring</td>
</tr>
<tr>
<td>ARH/VRQ</td>
<td></td>
</tr>
<tr>
<td>AHQ/VRQ</td>
<td></td>
</tr>
<tr>
<td>VRQ/VRQ</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Sheep PrP genotypes and risk of scrapie

Information adapted from the DEFRA National Scrapie Plan for Great Britain, Ram genotyping scheme.
that the UK NSP breeding programme will markedly reduce the risk of TSE infection by producing higher frequencies of ARR alleles in the sheep population.

Has BSE infected sheep naturally?

Since it is known that BSE can infect sheep in laboratory conditions and sheep on farms are on occasion fed nutritional supplements which at one time contained meat and bone meal, it is theoretically possible that BSE has infected the UK sheep flock in a similar manner to UK cattle. Several studies have analysed the small amount of data available and have concluded that, if BSE is present in UK sheep, it is at a very low level\textsuperscript{29–31}. However, it is obviously important to understand how BSE behaves in sheep in case this hypothetical scenario becomes real. Alarmingly, most of the studies to date have shown little difference between BSE and scrapie in sheep in terms of distribution of infection throughout the body, clinical signs and pathology\textsuperscript{31}. The one sure method of identifying BSE is by its transmission characteristics in a specified panel of mouse strains, but this can take up to 2 years to produce a result\textsuperscript{6,32}. Therefore, other means are being sought to distinguish harmless scrapie from harmful BSE. In terms of legislation, the UK approach of total eradication of all TSEs in sheep may be required in order to make sure of removing any risk of BSE infecting humans eating sheep meat or milk products.

Pathology

The major lesions associated with TSEs are the spongiform changes associated with neurones and visible under the light microscope as holes in the tissue sections, known as vacuolation. In mice, the patterns of vacuolation of different brain areas (lesion profile) is dependent on the strain of scrapie or BSE with which the animal has been infected and this is one of the reasons that it is known that vCJD in humans is caused by the same infectious agent as BSE in cattle\textsuperscript{32}. However, in sheep, it seems that similar lesion profiling will not work as a strain identifier as it is too variable\textsuperscript{33}.

As deposition of the disease-associated PrP\textsuperscript{Sc} protein has also been found to vary in pattern in different mouse/scrapie strain combinations\textsuperscript{34}, a similar study was carried out in field cases of scrapie in sheep using immunohistochemistry. Brains of 43 sheep with clinical signs compatible with scrapie revealed 12 different PrP\textsuperscript{Sc} types based on examination of 8 different brain regions and giving rise to a PrP\textsuperscript{Sc}
profile\textsuperscript{35}. This is a complex analysis, but has shown promise and may have potential in screening \textit{post mortem} for sheep which are infected with BSE.

**Biochemical analysis (glycoform studies)**

If sheep are to be used to provide food for human beings, a technically complex \textit{post mortem} test may be too slow to provide a reliable answer as to whether the carcass is safe to eat. Currently being exploited in cattle is a series of methods based on the detection of disease-associated PrP\textsuperscript{Sc} protein in brain using methods which take only a few hours to provide a result.

The PrP protein molecule has two sites at which carbohydrates can be attached. The mature protein, therefore, exists in 4 different forms, or glycoforms, where both sites (diglycosylated), either one of two single sites (monoglycosylated) or no sites (unglycosylated) are occupied. This gives rise to a pattern on Western blots which has been said to be characteristic of different strains of TSEs\textsuperscript{36}. Although this remains unlikely to be useful in natural sheep scrapie which appears to be remarkably invariant in pattern\textsuperscript{37,38}, it is true that BSE (and vCJD in humans) does have a distinct pattern tending to have higher concentrations of the diglycosylated form of the molecule than are usually seen with other strains (Fig. 1)\textsuperscript{39}.

In TSE affected sheep, it has been shown that PrP\textsuperscript{Sc} protein is not only deposited in the central nervous system but is also found in peripheral tissue, such as spleen and lymph nodes. This finding has been exploited

![Western blot of disease-associated PrP\textsuperscript{Sc} protein showing glycoform pattern differences.](https://academic.oup.com/bmb/article-abstract/66/1/171/284808)
to provide a live-animal test for infection using sampling from tonsils\textsuperscript{40} or from the nictitating membrane (third eyelid with associated lymphoid tissue) of the sheep’s eye\textsuperscript{41}. In experimental studies, these tests are useful because PrP\textsuperscript{Sc} protein is detectable during the early, pre-clinical phase of infection. For example, in sheep which went on to develop natural scrapie at about 2 years of age, one group reported being able to find PrP\textsuperscript{Sc} in tonsil biopsies taken as early as 3 months of age\textsuperscript{42}. Such a test, if proved to be reliable and accurate, would be immensely useful also in the food industry.

**Problems with screening methods**

Although tests based on detection of PrP\textsuperscript{Sc} show great promise, detailed pathogenesis studies in sheep have shown that the precise tissues which accumulate detectable levels of PrP\textsuperscript{Sc} depend on the PrP genotype of the animal. In one study\textsuperscript{16} using sheep experimentally infected with scrapie, disease developed in VRQ/VRQ, VRQ/ARQ and VRQ/ARR animals. Protein analysis of various body tissues showed clearly that VRQ/VRQ animals had signs of infection (PrP\textsuperscript{Sc}) throughout the body in all lymph nodes examined. In the heterozygote sheep, this was not the case: in some individuals no PrP\textsuperscript{Sc} was detected in lymph nodes even at terminal clinical stages, whereas in others the presence of the protein was highly variable and, therefore, unreliable. Similar findings have been reported by other groups studying natural scrapie\textsuperscript{43}. Therefore, in imposing any kind of bioassay testing of sheep prior to slaughter, although a positive result would be reliable, a negative result would not provide the total safety from infection required for entry into the human food chain. *Post mortem* brain analysis remains, at the moment, the only safe possibility.

**Use of the sheep model in studies on the safety of blood transfusion**

Amongst many sources of concern about the human disease vCJD, one major question relates to the safety of blood transfusions and blood products – especially when inadvertently sourced from individuals during the long pre-clinical phase of vCJD, when they may act as asymptomatic carriers of the infectious agent. There is no epidemiological evidence to indicate that iatrogenic CJD has ever occurred via blood or blood products, but vCJD is a new disease with different pathogenesis, involving infection of peripheral lymph nodes (unlike CJD which does not) and may present different risks\textsuperscript{44,45}.

Sheep infected orally with BSE show shortest incubation periods in the
genotype ARQ/ARQ and these sheep show wide-spread deposition of PrPSc in the lymphoreticular system similar to that seen in human vCJD patients. In contrast, in human sporadic CJD and cattle BSE cases, the peripheral pathogenesis does not appear to involve the lymphoid system. Sheep were chosen as a model in which to study transmission of TSEs by blood transfusion, because of the similarity of the pathogenesis with vCJD and because large volumes of blood can be transferred in the absence of a species difference which is known to have an effect on transmission of TSEs.

In this study, 24 transfusions from BSE-challenged sheep at clinical and pre-clinical phases were carried out, including 7 with buffy coat preparations and 17 with whole blood. The transfusion recipients were BSE-susceptible sheep of New Zealand (scrapie-free) origin, thus avoiding any complications of scrapie infection in the background. Four of the 24 recipients have so far have been culled after showing clinical signs typical of TSEs in sheep. Two of these were confirmed at the time of writing by histopathology, immunocytochemistry and Western blotting for the presence of the BSE-like glycoform pattern in PrPSc protein. The other two await confirmation but are likely to be genuine. The confirmed cases (D505 and F19) occurred 610 and 538 days, respectively, following transfusion with whole blood taken from donor sheep both approximately at 50% of their incubation periods with BSE and, therefore, pre-clinical and apparently healthy. The two additional sheep (still to be confirmed biochemically) were culled with clinical signs of BSE having been transfused with whole blood taken when the donors were themselves at BSE clinical phase. The remaining transfused sheep are so far also healthy, but not all have reached the 500–600 days post-transfusion when the other recipients became sick. If the BSE suspect transfusion cases are confirmed, this brings the minimum rate of infection by transfusion in this part of the study to 4 out of 24, or roughly 17%. In a parallel experiment, sheep which were known to be susceptible to developing natural scrapie were used as blood donors and successful transmission of scrapie by this route has also been achieved in 4 cases out of 21 transfusions so far.

The results to date indicate that, with more than 10% of transfusions resulting in disease in the recipients, blood transfusion, even at pre-clinical stages, represents an appreciable risk for transmission of TSEs in sheep and, by extension, of vCJD in human beings. The relatively short and consistent incubation periods seen in the sheep-positive transfusion cases suggests that levels of infectivity in blood may be quite high, even in the pre-clinical stages of infection and/or that transmission by the intravenous route is highly efficient. The current legislation which is aimed at protecting the UK population from vCJD infection by use of leukocyte-depleted blood can clearly be seen to have been fully justified.
Future implications

A great deal has been learned about sheep TSEs in the last 15 years, much of it dependent on the PrP gene and protein – also true for the human equivalent diseases. The complex nature of the sheep disease, although it may be difficult to understand and explain the genetics and pathogenesis, sufficiently resembles the complexity of the human TSE diseases for sheep to be used as a model system for studying common underlying mechanisms in large mammals in a manner impossible in humans. Examples of high priority research projects include:

1 Therapeutics. The sheep/scrapie model is ideal for testing potential therapeutic agents in a natural infection. Sheep known to be at high risk of scrapie because of their genotype and their exposure to infection in a flock with endemic scrapie will be treated with drugs (e.g. pentosan polysulphate) which mouse studies suggest might delay disease onset, and observed for differing survival times compared with untreated animals.

2 Safety of blood products. BSE infection in the blood of experimentally challenged sheep can be detected by transfusion into other susceptible sheep; therefore, it should be possible to detect infectivity (or a surrogate marker) by biochemical means. Knowledge of which cell type harbours infection would enable filtering and cleaning strategies for safe use of UK blood products and would also aid diagnosis and detection of TSEs to protect human and animal health.

Acknowledgement

Figure 1 is reproduced courtesy of Angela Chong, (Neuropathogenesis Unit, Institute of Animal Health, Edinburgh, UK).

References

5 Foster JD, Parnham D, Chong A, Goldmann W, Hunter N. Clinical signs, histopathology and genetics of experimental transmission of BSE and natural scrapie to sheep and goats. Vet Record 2001; 148: 165–71
6 Bruce ME, Boyle A, Cousens S et al. Strain characterization of natural sheep scrapie and comparison with BSE. J Gen Virol 2002; 83: 695–704
10 Dickinson AG, Stamp JT, Renwick CC. Maternal and lateral transmission of scrapie in sheep. J Comp Pathol 1974; XX: 84
12 Foster JD, McKelvey WA, Mylne MJ et al. Studies on maternal transmission of scrapie in sheep by embryo transfer. Vet Rec 1992; 130: 341–3
26 Hunter N, Cairns D. Scrapie-free Merino and Poll Dorset sheep from Australia and New Zealand have normal frequencies of scrapie-susceptible PrP genotypes. J Gen Virol 1998; 79: 2079–82
Scrapie and experimental BSE in sheep

36 Hill AF, Desbruslais M, Joiner S et al. The same prion strain causes vCJD and BSE. Nature 1997; 389: 448–50
42 Schreuder BEC, vanKeulen LJM, Vromans MEW, Langeveld JPM, Smits MA. Tonsillar biopsy and PrPSc detection in the preclinical diagnosis of scrapie. Vet Record 1998; 142: 564–8
46 Foster JD, Parnham DW, Hunter N, Bruce M. Distribution of the prion protein in sheep terminally affected with BSE following experimental oral transmission. J Gen Virol 2001; 82: 2319–26
50 Farquhar C, Dickinson A, Bruce M. Prophylactic potential of pentosan polysulphate in transmissible spongiform encephalopathies. Lancet 1999; 353: 117