EUROPEAN COMMISSION
DIRECTORATE-GENERAL XXIV
CONSUMER POLICY AND CONSUMER HEALTH PROTECTION
Scientific Health Opinions

SCIENTIFIC OPINION ON

THE POLICY OF BREEDING AND GENOTYPING OF SHEEP, I.E. THE ISSUE OF WHETHER SHEEP SHOULD BE BRED TO BE RESISTANT TO SCRAPIE

ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 22-23 JULY 1999.

OPINION

The Scientific Committee Animal Health and Animal Welfare (SCAHAW) raised the attention of the Commission on the circumstances under which scrapie infection can be spread "silently" and on the question whether genetic factors increase the incubation period before sheep have had time to develop the disease. It recommended that further developments on genotyping and the role of persistent infectivity in sheep populations should be taken into consideration.

The Commission therefore invited the Scientific Steering Committee (SSC) to provide its scientific opinion on this issue. Starting point should be the following statement: "*The Commission noted that there was a need for advice on the policy of breeding and genotyping of sheep, ie whether sheep should be bred to be resistant to scrapie with the risk of producing more silent cases of the infection or whether they should be bred to be susceptible which would make the disease more evident*"

More precisely, the Scientific Steering Committee was requested to address the following 6 questions:

1. What is the risk that flocks of scrapie resistant sheep would carry the scrapie agent without showing clinical signs but at the same time being able to transmit the agent horizontally, vertically or via rendering?
2. Would this risk be reduced, and to which degree, if scrapie sensitive sheep would be included into resistant flocks as indicator animals? How many indicator animals would be needed?
3. Would the risk of undiscovered scrapie be further reduced by flocks only composed of sentinel susceptible sheep?
4. How could the (possible future) wide-scale introduction of scrapie-resistant sheep affect the monitoring of scrapie-infection?
5. The possible breeding and subsequent introduction of resistant sheep is likely to take many years. It is expected that in the (near?) future, tests for the diagnosis of scrapie on live animals will become available. Would the application of such tests and the subsequent culling of all positive sheep, eventually lead to the eradication of scrapie and consist of an alternative for the introduction of scrapie resistant breeds?
6. The theoretical possibility of BSE occurring in sheep: would the risks be similar to scrapie?

A special Working Group was created to address these questions.

The Scientific Steering Committee refers to the Discussion section in the attached report of the Working Group, which deals in detail with these issues.

In spite of some uncertainties, highlighted in the Working Group's answers to the above questions, the SSC concludes that the possibility that sheep may harbour a latent scrapie infection exists and if so, that they could pass an infection to other sheep. It is however expected that the background levels of infection in a resistant sheep population are significantly lower than in a susceptible population. There are firm indications that the scrapie agent, once introduced in an area, can persist in the environment and that maternal and horizontal transmission contributes significantly to the spread within a susceptible sheep population. Therefore the use of indicator animals or flocks only composed of sentinel susceptible sheep could increase the infective load in the environment and thus jeopardise the possibility to control and eventually eradicate scrapie. If BSE would have infected sheep, which is not certain today, and if BSE transmits and behaves in sheep in a manner similar to scrapie, for which there are some experimental indications, then a similar strategy for BSE and scrapie should be adopted.

The SSC recommends the following steps as a preliminary to the introduction or steering in more E.U.-countries of scrapie-resistance breeding programmes:

- At the level of the EU: analysis and evaluation of the breeding programmes that are presently ongoing in a number of European countries (for example: France, The Netherlands, the United Kingdom, Norway, Sweden, …[1]); establishment of a register with the resistant breeds per country and their corresponding genotypes.
- At the level of each EU country: start the genotyping of large numbers of animals, in order to acquire a view on the distribution of the various genotypes in the national flocks. This would provide a first step towards the estimation of the distribution of genotypes involved in resistance against scrapie and, possibly, BSE.

The SSC further recommends that, following the acquisition of these data, strong consideration should be given to the use of appropriate resistant strains of sheep, coupled with the development of and extensive use of validated diagnostic tests.

Before embarking on large scale breeding of sheep towards maximal resistant genotypes, according to the different breeds involved, consideration should be given to phenotypic characteristics.

[1] see also the attached Working Group report

REPORT FROM THE WORKING GROUP

1. General Mandate

The Scientific Committee Animal Health and Animal Welfare (SCAHAW) raised the attention of the Commission on the circumstances under which scrapie infection can be spread "silently" and on the question whether genetic factors increase the incubation period before sheep have had time to develop the disease. It recommended that further developments on genotyping and the role of persistent infectivity in sheep populations should be taken into consideration.

The Commission therefore invited the Scientific Steering Committee (SSC) to provide its scientific opinion on this issue. Starting point should be the following statement: "*The Commission noted that there was a need for advice on the policy of breeding and genotyping of sheep, ie whether sheep should be bred to be resistant to scrapie with the risk of producing more silent cases of the infection or whether they should be bred to be susceptible which would make the disease more evident*"

The SSC subsequently established a Working Group with the following specific mandate:

1. What is the risk that flocks of scrapie resistant sheep would carry the scrapie agent without showing clinical signs but at the same time being able to transmit the agent horizontally, vertically or via rendering?
2. Would this risk be reduced, and to which degree, if scrapie sensitive sheep would be included into resistant herds as indicator animals? How many indicator animals would be needed?
3. Would the risk of undiscovered scrapie be further reduced by flocks only composed of sentinel susceptible sheep?
4. How could the (possible future) wide-scale introduction of scrapie-resistant sheep affect the monitoring of scrapie-infection?
5. The possible breeding and subsequent introduction of resistant sheep is likely to take many years. It is expected that in the (near?) future, tests for the diagnosis of scrapie on live animals will become available. Would the application of such tests and the subsequent culling of all positive sheep, eventually lead to the eradication of scrapie and consist of an alternative for the introduction of scrapie resistant breeds?
6. In addressing these questions the SSC should also discuss the theoretical possibility of BSE occurring in sheep. Would the risk be similar?

The Working Group submitted the report hereafter to TSE/BSE *ad-hoc* group.

2. INTRODUCTION AND BACKGROUND

2.1. Definitions

PrP^{C}	prion protein, the normal form
PrP^{Sc}	prion protein, the disease-associated form, not used herein to indicate infectivity
PrP gene	the sheep PrP protein encoding gene
BSE	bovine spongiform encephakopathy
TSE	transmissible spongiform encephalopathy, an alternative, less controversial, name for prion diseases
Infection	scrapie infection, scrapie agent, to be considered separately from PrP^{Sc}
Susceptible sheep	animal with PrP genotype known to predispose to scrapie
Resistant sheep	animal with PrP genotype known to remain clinically free of scrapie signs for normal lifespan
Carrier	animal with latent scrapie infection, that will not itself develop disease but with the ability to pass infection on to other sheep

2.2. Genetics of incidence of natural scrapie

Studies of natural scrapie in sheep have confirmed the importance of three amino acid codons in the sheep PrP gene (136,154 and 171) (Belt *et al*, 1995; Clouscard *et al*, 1995; Hunter *et al*, 1996) originally shown to be associated with differing incubation periods following experimental challenge of sheep with different sources of scrapie and BSE (Goldmann et al. 1991; Goldmann et al. 1994). The three polymorphic amino acids are shown in **Table 1**. Any particular combination of three codons is known as an allele but not all theoretically possible alleles have been found.

There are breed differences in the frequencies of the occurrence of PrP alleles and in the exact disease-associated alleles, however some clear genetic rules have emerged. (The PrP genotype (given by specifying both alleles) of a sheep is described listing in order codons 136, 154 and 171 for each allele in turn.) Using this scheme, the most resistant sheep PrP genotype is ARR/ARR. Out of hundreds of scrapie affected sheep studied world wide, only one animal of this genotype has been reported with clinical scrapie - a Japanese Suffolk sheep (Ikeda *et al*, 1995) - and as more and more scrapie sheep are genotyped, the significance of this single Japanese finding becomes less and less. Sheep of ARR/ARR genotype are also resistant to experimental injection with both scrapie and BSE (Goldmann *et al*, 1994).

The simplest UK breeds in terms of PrP genetics are Cotswold, Hampshire Down, Soay, Suffolk, and Vendeen which all normally encode just two PrP alleles: ARQ and ARR, although ARH is sometimes also found in some flocks of these breeds. Taking Suffolks as the paradigm breed for this group, Suffolk sheep of genotypes which encode Q on both alleles at codon 171 are most susceptible to scrapie. For example, many ARQ/ARQ Suffolks develop scrapie (**Table 2a**), although many also remain healthy (Westaway *et al*, 1994; Hunter *et al*, 1997). Occasionally scrapie occurs in ARQ/ARR sheep but has never in the UK been found in ARR/ARR animals.

Most UK sheep breeds are much more complex in PrP genetics than the Suffolk-type group, especially those breeds which encode the VRQ allele. For example Cheviot, Swaledale and Shetland sheep have 4 PrP gene alleles: VRQ, ARQ, ARR, and AHQ. Sheep with the genotype VRQ/VRQ appear to be extremely susceptible to scrapie (**Table 2b**) (Hunter *et al*,

1994; Hunter et al. 1996). Most complicated of all, are Texels and Lleyn sheep which have 5 PrP alleles and 15 genotypes. In the latter breeds, scrapie usually occurs in sheep of VRQ/ARQ, VRQ/ARH and VRQ/VRQ but is found occasionally in 5 other genotypes.

Table 1: PrP gene alleles and polymorphic amino acid codons

Amino acid number	Codon	Single letter code	Allele*
136	valine	V	VRQ
	alanine	A	ARQ, AHQ, ARR
154	arginine	R	ARR
	histidine	H	AHQ
171	arginine	R	ARR
	glutamine	Q	VRQ, ARQ, AHQ

* most codons are found on only one allele, some on three alleles. Alleles are given with codon 136, 154 and 171 in order.

Table 2: Suffolk and Cheviot sheep PrP genotypes and natural scrapie

a) **Suffolk sheep:**

PrP Genotype	Natural scrapie
ARQ/ARQ	High risk of scrapie
ARQ/ARR	Occasional occurrence
ARR/ARR	Resistant

b) **Cheviot sheep:**

PrP Genotype	Natural scrapie
VRQ/VRQ	Very high risk of scrapie
VRQ/ARQ	Very high risk of scrapie
VRQ/ARR	Occasional occurrence
ARQ/ARQ	Resistant
ARQ/ARR	Resistant
ARR/ARR	Resistant

Many other studies of sheep throughout Europe (both EU and non-EU countries) and the USA confirm and extend these findings of both genetically simple breeds at one extreme and complex breeds at the other (Laplanche *et al*, 1993; Westaway *et al*, 1994; Belt *et al*, 1995; Belt *et al*, 1995; Clouscard *et al*, 1995; Ikeda *et al*, 1995; Bossers *et al*, 1996; Elsen *et al*, 1996; O'Rourke *et al*, 1996 ; Elsen *et al*, 1997; Junghans *et al*, 1998, Thorgeirsdottir *et al*, 1998; Elsen *et al*, 1999; Tranulis *et al*, 1999). Within the simple breeds, like Suffolks, breeding for resistance involves the selection of ARR/ARR rams, there is little other choice. For more complex breeds of sheep, like Cheviots, there is much more choice in the range of relatively resistant genotypes. However in the UK, commercial testing for PrP genotype has resulted in the premium status of ARR/ARR animals of any breed and selection only for this genotype. The potential effects of such enthusiastic selection are under study at the moment in a large scale sheep epidemiology project in the UK headed by Angela McLean of the Biotechnology and Biological Sciences Research Council (BBSRC), Institute for Animal Health (IAH) Further relevant studies include: a EU network co-ordinated by JM Elsen at the Institut National de Recherche Agronomique (INRA) (CT97/3305) with 17 partner laboratories is looking at the genetics of scrapie incidence in many EU countries and in

improving diagnostic tests, CT98/6056 (Lantier, INRA) is setting up a European scrapie network on epidemiological databases and biological sample banks, and CT98/7017 (Elsen, INRA) is looking for the influence of genes other than PrP in scrapie control. In the Netherlands, a ten year scrapie control programme is underway using PrP genotype to reduce genetic susceptibility to disease and if possible to eliminate scrapie in the entire sheep population of the country. In approximately 15 flocks, the anticipated reduction in incidence of scrapie in relation with the changes in genotypes, will be closely monitored. (Schreuder, pers. comm.). Also in Norway, a 5-year closed 10-flock breeding project has started in which all sorts of phenotypic characteristics (live weights gains, slaughter quality, wool quality, fertility, disease occurrence etc.) are being monitored as the animals are bred towards ARR/ARR genotypes (Ulvund, pers.comm). Such control programmes are not limited to Europe as in Michigan, USA, genotype screening is to be used to attempt to establish 171 RR homozygotes and remove all 171 QQ homozygotes in the black-faced sheep population. (Detwiler, pers. comm.)

2.3. Experimental scrapie studies and strains.

A series of experimental challenge experiments has been carried out by IAH scientists using (NPU) Cheviot sheep bred over many decades into two lines differing in response to scrapie. Following challenge by subcutaneous injection, the scrapie source SSBP/1 affects Cheviot sheep according to their codon 136 genotype causing disease only in sheep encoding at least one VRQ allele. In contrast, both the scrapie source CH1641 and experimental BSE target sheep encoding Q at codon 171 on both PrP gene alleles and producing disease with shortest incubation period in sheep which are ARQ/ARQ (Goldmann *et al*,. 1994). It is possible therefore that there are also various types or strains of natural scrapie which target either particular sheep breeds and/or different PrP codons and could explain why some sheep flocks differ in the precise details of the genotypes of sheep which do and do not develop scrapie. For example one flock of Poll Dorset sheep is affected by scrapie which apparently targets sheep according to codon 171 genotype, equally affecting animals which are VRQ/VRQ, VRQ/ARQ and ARQ/ARQ. A different Poll Dorset flock has shown scrapie cases only in VRQ/VRQ and VRQ/ARQ genotypes despite ARQ/ARQ being a frequent genotype in the flock (Hunter *et al*, 1997 and unpublished).

There is also extensive evidence for different strains of TSE in laboratory mice (Bruce *et al*, 1991; Kuczius and Groschup, 1999) and hamsters (Caughey *et al*, 1998) and also in humans (Bruce *et al*, 1997). It is therefore very likely that there are at least a few different strains of sheep scrapie in the sheep flocks of the EU and that these may target particular PrP genotypes. However, on the basis of the PrP genotypes of sheep affected by scrapie, these are remarkably similar throughout the world and may mean that there is actually relatively little variation in scrapie strains. Strains are differentiated on the basis of their transmission characteristics, a process which is both expensive and time consuming as resulys are often not available for around 2 years. Increasingly, strains are being characterised on the basis of the appearance of PrP^{Sc} protein on Western blots. PrP^{Sc} is relatively protease resistant and produces a three-banded pattern which is related to the conformation of the protein. This is generally called glycoform analysis and is being thoroughly investigated by many EU laboratories for its usefulness in screening samples for possible BSE infection on which point there is some disagreement (Hill *et al*, 1998; Hope *et al*, 1999). In one study, an experimental scrapie source CH1641 gave a similar PrP^{Sc} glycoform pattern to that seen with experimental BSE-infected sheep (Hope *et al*, 1999) so there is a great potential problem over confusion of BSE with scrapie biochemically. It has been reported that the examination of sheep scrapie samples (brain, spleen and placenta) may produce different patterns from the same individual animal. However, as experimental details may have an impact on the

resulting patterns, further thorough investigations aiming at the standardisation of this technique are necessary before any further conclusions should be drawn. The PrP pattern may be giving an indirect indication that there are indeed different natural scrapie strains causing disease. At present, however, it would be premature to rely on glycoform analysis for distinguishing scrapie strains or for distinguishing scrapie from BSE.

2.4. Pathogenesis and infectivity

Scrapie is usual diagnosed post-mortem on the basis of clinical signs, histopathology and, increasingly now, detection of PrP^{Sc} protein on Western blots, immunohistochemistry or in the form of scrapie associated fibrils (SAF). It is perhaps wise to make use of as many such tests as possible before arriving at a definite diagnosis. There are examples of both negative histopathology with either no or inconclusive numbers of vacuolated neurons and as such changes can be caused by diseases and disorders other than scrapie, it is important to be sure that a TSE is really indicated. There is an urgent need to standardise the means by which a TSE positive diagnosis is reached.

The annex (taken from the text of the document produced by the EU Working Group on The risk of infection of sheep and goats with BSE agent.) lists sheep and goat tissues which have been tested for infectivity by mouse bioassay and is taken from the published work of Hadlow (Hadlow *et al*, 1979; Hadlow *et al*, 1980; Hadlow *et al*, 1982), Pattison (Pattison, 1964; Pattison *et al*, 1972) and Groschup (Groschup *et al*, 1996). These are therefore the tissues which would be of interest when considering where a hidden TSE infection might exist in an apparently healthy, or even resistant, sheep. It is not known for certain whether resistant sheep are capable of replicating infectivity at a low level or whether such animals also have the ability to spread infection to other sheep. The former need not imply the latter.

There are a few studies which have looked at tissues amenable to sampling while the animal is still alive. For example, in susceptible sheep, biochemical evidence of infectivity (PrP^{Sc}) can be found at early stages of the incubation period in tonsil and other lymphoreticular tissues (Schreuder *et al*, 1996; Schreuder *et al*, 1998). Another potentially useful finding is that the nictitating membrane (third eyelid) lymphoid tissue of sheep also accumulates PrP^{Sc} during the pre-clinical phase of scrapie (O'Rourke *et al*,. 1998). This is an easily sampled tissue and, as with tonsil biopsy, third eyelid biopsy is being investigated on a large scale to assess its reliability in diagnosis in individual sheep. A further tissue which may prove interesting, and one which is greatly implicated in the contagious spread of scrapie between sheep, is the placenta. There is evidence that scrapie can be transmitted from sheep placental tissue samples (Pattison *et al*, 1972; Onodera *et al*, 1993) but there have also been transmission failures. To add to the uncertainty about the infectiousness of placenta a recent study, although finding a good correlation between infectivity and PrP^{Sc} distribution, found that it was possible, over two pregnancies in the same individual animal, for the first placenta to be PrP^{Sc} positive and the second to be PrP^{Sc} negative (Race et al. 1998). The two sheep that gave rise to the latter result did not have the PrP^{Sc} negative placentas tested by bioassay for infectivity so it may be simply that infection is limited to specific parts of the placenta, missed in the second samples. Placental sampling on a flock basis may be useful in showing whether infection is present within a flock. Resistant sheep in such studies are generally PrP^{Sc} negative, however it is not certain whether this is a genuine and complete negative or whether it means that PrP^{Sc} levels are below the current levels of detection. Perhaps the resistance to clinical signs and/or to infection is rather relative and not an absolute feature.

It is possible that PrP genotype, route of infection or strain of infecting TSE agent may influence the tissue distribution of both infectivity and PrP^{Sc} (Somerville *et al*, 1997; Ulvund *et al*, 1998) and this requires further study with the new and very sensitive methods of

detecting PrP^{Sc} which are being developed by several laboratories (Hope, personal communication, (Safar *et al*, 1998)) using antibodies which do not differentiate between PrP^{Sc} and PrP^{C} and relying on the difference in protease sensitivity to tell them apart. However care is required in the interpretation of results as the differentiation between the protease resistance of PrP^{Sc} and the sensitivity of PrP^{C} is not as absolute as many people think. Even PrP^{C} has some residual protease resistance (Buschmann *et al*, 1998) and conditions have to be defined carefully before accepting a low level of PrP^{Sc} as a positive infectivity marker. A new antibody which does distinguish PrP^{Sc} from PrP^{C} is not yet generally available in commercial detection kits (Korth *et al*, 1997).

2.5. Maternal and Contagious Spread

There are many ideas about how scrapie is spread from sheep to sheep. This may occur directly or via some other environmental reservoir of infection such as hay mites (Wisniewski *et al*, 1996) or nematodes (Clouscard *et al*, 1995) and these factors are being further investigated in PL98/7023 co-ordinated by L.Gruner, INRA.

In the study of the sheep itself as a direct source of natural scrapie transmission to other sheep, it is extremely difficult to ensure that the test susceptible animals come from scrapie free environments and are not already harbouring infection. Observation is necessary over extremely long timescales to be certain of picking up all possible cases. Two large scale studies go a long way towards fulfilling the exacting controls required and suggested that both maternal and contagious transmission may occur. Both studies were prior to the discovery of PrP genetics and its importance in control of scrapie incidence. In one of these carried out in Scotland, (Dickinson *et al*, 1974) Scottish Blackface sheep were used as the indicator animals because no scrapie case had been reported in this breed for the previous thirty years. It was known to be possible to infect Blackface sheep by inoculation however (Gordon 1946). The source flock was kept on a remote hill farm and observed for thirteen years during which time no case of scrapie was found in a total of over 18 000 animals. Seventy five sheep were taken away from this flock and mixed with scrapie sheep (Suffolk x Blackface originating from a high incidence Suffolk flock at the Moredun Research Institute, Edinburgh) in normal field conditions in an area not previously used for scrapie studies. The incidence of scrapie in the indicator animals was 28% in 5 - 6 years. This level in the source Blackface flock would have given rise to over 200 scrapie cases in this time and so was regarded as highly significant. All suspect cases were confirmed by histopathology and rejected if negative. Maternal transmission was also found to be important in this study, the scrapie status of the mother being much more important than that of the father in predicting which lambs would ultimately succumb. However in more recent computer modelling shows that even if 100% of vertical transmission was to be eliminated, only a very small reduction in the number of cases per year would be achieved (Woolhouse *et al*, 1998) .

Hourrigan (Hourrigan *et al*, 1979) carried out a field trial (using extremely stringent controls) to establish the possibility of horizontal scrapie transmission in l964 at Mission in Texas. Sheep judged to be scrapie-free were run in field conditions with sheep known to have been exposed to the disease. A diagnosis of scrapie was in all cases confirmed by histopathological examination of brain and/or transmission to mice. A scrapie-free label was only assigned after observation for 8 years after bringing sheep into the programme at 2 years of age. Animals dying were examined carefully for any signs of scrapie and histopathology was carried out. Any animals over which there was some doubt either clinically or pathologically were excluded[2] from the final report. The exposed animals brought into the field station (547 in number) were known to be from scrapie flocks but were

[2] It would be useful to know whether the numbers of such sheep were sufficient to introduce possible biases .

apparently healthy. The breeds were Cheviots, Hampshire, Montadale, and Suffolk in 21 blood lines. The unexposed group consisted of the following: 31 Targhee and 31 Rambouillet (no scrapie case reported in the US in either of these breeds although Rambouillet sheep are credited with bringing scrapie into North India - (Zlotnik and Katiyar 1961)), 33 Hampshire, 28 Suffolks[3] and 17 goats. The previously exposed group included 333 Suffolks and 29% of these developed scrapie. Their progeny, bred at the station numbered 446 of which 34% later came down with scrapie. Of the unexposed indicator animals, five (3.5%) contracted scrapie and all at the later than average age of 6 -8 years. The progeny of the whole flock, exposed from birth to infection came down with scrapie at the rate of 27% at the more usual age range of 2.5 - 5 years. Although the incidence of five animals contracting scrapie out of a total of 140 (123 sheep and 17 goats) can be criticised, their late age of onset was argued to indicate that they were not exposed to scrapie before coming to the field station. However unlike the Dickinson study, the source flocks were not followed up and the "scrapie-free" label is more difficult to support.

A number of studies indicate that in close penning conditions, scrapie can be transmitted laterally (e.g., Brotherston *et al*, 1968; Dickinson *et al*, 1974). These have been criticised on the grounds that the conditions are not similar enough to those in the field where sheep will be much more spread out. Icelandic farmers might disagree with this however because, as reported by (Palsson, 1979), it is normal practice for them to keep their sheep in close penning conditions throughout the 5 - 6 months of winter. Icelandic scrapie, or rida, is not treated with the secrecy common elsewhere and, being openly acknowledged, is easier to study. Palsson believed that the winter conditions were more important in the spread of rida from sheep to sheep than pasture conditions and also claimed evidence of sheep in the preclinical state being a source of infection to their flockmates.

These studies, none of which is conclusive but all of which involved very long term observation and a lot of sheep, serve to indicate the difficulty of being certain of the origin of scrapie when it appears in susceptible animals. Pre-clinical scrapie could circulate for several years before the first case is seen and this is relevant to the anecdotal evidence frequently quoted that scrapie appears suddenly and "out of the blue" in sheep flocks. Lack of good surveillance may also contribute to this phenomenon.

In eastern Norway, where scrapie never has been diagnosed before, scrapie was diagnosed in 1998 in a 6 years old AHQ/AHQ sheep (spel), and within that flock there were three animals with the susceptible VRQ/ARQ genotypes, two of these were 5 years old and one was 6 years old (*Tranulis et al*, 1999). The finding of healthy, old and supposedly scrapie-susceptible animals in a flock must therefore be evaluated with great care when it comes to classifying the flock as scrapie free. This also has relevance concerning the significance of introducing sentinel susceptible sheep in flocks**.**

2.6. Routes of Transmission

The route of transmission of natural scrapie is not known. However extensive studies of the spread of infection within flocks of naturally or experimentally infected animals have suggested possibilities.

The high incidence Suffolk sheep of Mission, Texas, have provided information on the body distribution of scrapie (Hadlow *et al*, 1982). Tissues taken from sheep of ages varying from newborn lambs to 8.5 years and known to be at high risk of developing scrapie naturally were tested by mouse bioassay for the presence of infectivity. Scrapie was detected in lymphatic tissues and, interestingly, intestine in clinically normal 10 - 14 month lambs

[3] Of the 28 unexposed Suffolks, 20 were from New Zealand and two of these developed scrapie.

although titres were generally low. Infectivity was first detected in the central nervous system in a clinically normal 25 month old ewe and by the time disease developed in nine animals at approximately 3.5 years of age, scrapie was present in the alimentary tract from the tonsil to the distal colon as well as in the spleen and central nervous system. Scrapie was not detected in other tissues such as heart, lung, kidney and skeletal muscle nor was it found in high risk sheep which remained clinically normal until 4.5 - 8.5 years of age.

Because of the apparent early involvement of the alimentary tract in this form of Suffolk sheep scrapie, an oral route of transmission was postulated however milk, colostrum and feaces were also tested (by mouse bioassay) by (Hourrigan *et al*,. 1979) and nothing was found. (Pattison *et al*, 1972; Pattison, 1974; Race *et al*, 1998) had already demonstrated that it was possible to produce scrapie in Herdwick sheep by feeding them placental material from scrapie affected Swaledale ewes but infection did not seem to be present in the foetus (Hourrigan *et al*,. 1979) and so the idea of oral transmission of scrapie via infected placentae contaminating pasture was put forward.

It is clear from Icelandic attempts to eradicate scrapie that the presence of scrapie-affected sheep may not be necessary for scrapie to persist in the environment. For example, the contamination of pasture as a source of infection has some supporting evidence from Icelandic scrapie (Palsson, 1979) and from the work of (Greig, 1940; Greig, 1950) and could be quite long lived because of scrapie's infamous resistance to inactivation. Skin abrasions are also possible natural entry points for scrapie. This route has not been developed experimentally although (Dickinson, 1976) quoting from the unpublished work of Wilson in the l950s said that it was possible to inoculate sheep successfully with scrapie using scarification.

2.7. Sheep as carriers of infection

It is a possibility that sheep may harbour a latent scrapie infection exist and if so, that they could pass on infection to other sheep. Carrier animals are defined as those which have a latent infection and also have the ability to pass on the infection to other sheep. Resistant sheep may not show signs of clinical scrapie but could still harbour the infectious agent, posing a threat by maintaining the infectious agent and creating a potential situation in which a new strain, capable of causing clinical scrapie could be selected. Even if a new strain did not appear, computer modelling predicts that infection could remain hidden in the flock for many decades (Woolhouse *et al*, 1998) threatening the health of any susceptible animals introduced into the flock. There is some limited experimental evidence for the existence of hidden infectivity. One of the earliest studies of scrapie in mice (Chandler, 1963), showed that incubation period was dose-dependent. In other words, incubation period lengthened as the amount of infectivity in the inoculum was reduced. At very low levels of scrapie infection, replication may take such a long time to build up, that disease does not develop within the animal's normal lifespan (Dickinson *et al*, 1975). Such a situation, if shown to exist in sheep (and as yet there is no proof of this) could lead to the maintenance of a low level of infection in a flock without any signs of symptoms. Such sheep could be shedding scrapie infectivity for many years and be a source of infection for their flockmates. More recent evidence supporting such a phenomenon comes from another mouse model (scrapie strain 87V injected intraperitoneally (ip) as a 1% brain homogenate into IM mice) where there is long term persistence of high scrapie titres in the mouse spleen without either accompanying replication in the brain or development of scrapie symptoms. This could be a mechanism by which carrier status animals can be produced (Collis and Kimberlin, 1985). Cross species persistence may also occur, hamster scrapie injected into mice does not produce disease but the hamster scrapie remains in brain and spleen of the mice and can be recovered in a form still able to infect hamsters (Race and Chesebro 1998).

Which PrP genotypes of sheep may be most likely to harbour hidden infection, whether or not they can pass it on to other animals? It is almost certain that sheep of the most susceptible genotypes, will carry infectivity within their bodies in the pre-clinical phase. However for animals which remain healthy throughout a natural lifespan, those genotypes of sheep which occasionally have scrapie cases could be considered as potential carriers of infection: ARQ/ARR Suffolks and VRQ/ARR Cheviots for example (**see Table 2**).

2.8. Epidemiology

A number of studies have begun recently (Woolhouse, Centre for Tropical Veterinary Medicine, CTVM, University of Edinburgh, McLean, IAH and Hoinville, Veterinary Laoratory Agencies (VLA) on the epidemiology of scrapie in UK sheep using for example mathematical modelling techniques to test the effect of variation of different parameters (eg horizontal/vertical transmission, infection load, lifespan) on the basic reproduction number (R_0). R_0 is the average number of secondary infections produced by one infected individual introduced into a susceptible population. For R_0 less than unity, each infection on average fails to reproduce itself and the number of infected individuals is expected to decline towards zero. If, however, R_0 is greater than unity, each infection on average more than replaces itself and the outbreak, at least initially, will escalate (Anderson and May 1991).

The first attempt to estimate R_0 for a scrapie outbreak has been made. The study animals were in the NPU Cheviot flock through which scrapie spread between 1970 and 1982 (Dickinson, 1974). R_0 was calculated for this particular outbreak and was found to be 3.9 (Matthews *et al*, 1999) i.e. each infected sheep was infecting around 4 other animals and the disease was therefore able to spread. It is important to repeat these calculations with other flocks and other breeds but this study gives a pointer to the potential number of infections within an affected flock.

The INRA Romanov flock is also a subject of intense study from the start of its scrapie outbreak in 1993 (Clouscard *et al*, 1995). Between April 1993 and may 1997, 1015 animals were exposed to infection and 304 animals died of scrapie. There was a major genetic (PrP gene) influence on susceptibility but there was also some evidence for maternal transmission of infection, at least post-natally, and some indication that the risk of maternal transmission might be influenced by the mother's genotype independent of scrapie status and lamb genotype (Elsen *et al*, 1999). All lambs were born naturally and spent the first 24 hours with their mothers before some were removed and fed artificially.

These studies all involve single flocks and the spread of scrapie within that flock. The spread of scrapie between flocks still needs to be addressed and there is little data on which to base analysis. Obvious risk factors which could be considered are movement of sheep between flocks and use of common pasture.

3. DISCUSSION

3.1. What is the risk that flocks of scrapie resistant sheep would carry the scrapie agent without showing clinical signs but at the same time being able to transmit the agent horizontally, vertically or via rendering?

There is not enough information available to provide an answer to this question. However experiments would help to provide answers.

Experimental difficulties

The difficulties of studying this point include the following. The resistant potential donors of infection would need to be at high risk of having picked up infection and this could not be guaranteed, even if the animals were taken from a scrapie affected flock. Experimentally challenged sheep may have to be used and a decision made about which TSE to use to infect the sheep. Each strain of infection may have different transmission characteristics. Resistant control sheep would be required, i.e. unexposed resistant sheep. How could this be guaranteed? Even using biopsy for PrP^{Sc} and assuming the test was negative, would not necessarily mean that the animal was infection free at sub-detectable levels and detectable levels of PrP^{Sc} on its own would not mean that the animal was capable of passing on infection to other sheep.

The susceptible recipients of the infection, the indicators or sentinels, would require to be scrapie free prior to their exposure to the resistant donors. This cannot be guaranteed although the best option would be to use sheep from New Zealand (NZ) which is believed to be scrapie free. Also, the animals would require feeding with guaranteed TSE-free feed.

Where would the experiments be undertaken? Susceptible sheep could pick up infection from the environment rather than the exposed resistant sheep. There would be no way to differentiate the two. Greater control over the environment would be assured by keeping sheep on concrete which could be decontaminated prior to the start of the experiment.

Suggested experiments

Mix in separate groups

(a) exposed susceptible sheep with scrapie free susceptible sheep

(b) exposed susceptible sheep with scrapie free resistant sheep

(c) exposed resistant sheep with scrapie free susceptible sheep

(d) exposed resistant sheep with scrapie free resistant sheep

Monitor for evidence of disease in all sheep for at least the average normal lifespan. Sample tissues for evidence of PrP^{Sc} at termination of study.

3.2. Would this risk be reduced, and to which degree, if scrapie sensitive sheep would be included into resistant flocks as indicator animals? How many indicator animals would be needed?

It is difficult to know what the point of this would be, given the difficulties of interpreting the results if scrapie was to appear in the indicator animals. Where did it come from? Was the infecting scrapie within the flock or the environment or was it already present within the indicators prior to introduction into the test flock? It would be difficult to decide when the environment of a flock which had once had scrapie would become clean of contaminating infection.

It needs discussion on how this question should be tackled, but in any case a period of experimental study would be necessary prior to adopting this as a control policy.

One may suggest a period of 10 years without clinical disease in a resistant flock may be suggested as a suitable time scale to wait prior to introduction of susceptible sentinels. NZ sheep could possibly be used for the indicator animals but this may not be popular with European farmers who use different breeds to those found in NZ. One may also suggest to have a central nucleus flock of susceptible scrapie free animals in a clean environment and to introduce resistant sheep from flocks wishing to establish their freedom from disease.

It is thought impossible at the moment to estimate how many animals would be needed to be sure of proving a negative result.

3.3. Would the risk of undiscovered scrapie be further reduced by flocks only composed of sentinel susceptible sheep?

The reasoning behind this suggestion is that scrapie would be revealed in a susceptible population easily and the affected flocks could be simply slaughtered - a stamping out policy. The SSC is against this proposal because of the long term nature of the scrapie incubation period.

The problem here is for how long a period can scrapie exist in such a flock before any sheep develop clinical signs. Because it is probable (but not yet certain) that sheep can transmit scrapie well before showing clinical signs, and because many infected sheep do not live long enough to show signs (so that infections may outnumber cases by 2:1 or more), this period could be several years.

This approach would lead to much higher levels of infectivity in the sheep population and possibly the environment than if a largely resistant population existed. Infectivity in resistant animals, should it exist at all, is expected to be at much lower, and therefore safer, levels than in even pre-clinical susceptible animals. The SSC therefore thinks that 100% susceptibility in the sheep population is not a good idea.

A minor point here is that the most susceptible sheep (VRQ/VRQ genotype) are rare and it would take an extremely long time to replace the present European sheep population with this genotype.

3.4. How could the (possible future) wide-scale introduction of scrapie-resistant sheep affect the monitoring of scrapie-infection?

The answer to this depends on the sensitivity of the diagnostics, if a carrier state exists and if it can be detected.

The use of PrP^{Sc} as a marker for infectivity will be uncertain until the sensitivity of all the available diagnostic tests is known and compared. The sensitivity may be affected by genotype, age, breed etc of the sheep. Extensive trialling of tests outside the controlled laboratory environment is required.

However it is felt that as resistant sheep would be expected to harbour lower levels of infection, any monitoring system based on PrP^{Sc} detection may be reduced in power as it would need to by highly sensitive. Being alert to the possibility of false negative results at the limits of the detection sensitivity would also be essential.

3.5. The possible breeding and subsequent introduction of resistant sheep is likely to take many years. It is expected that in the (near?) future, tests for the diagnosis of scrapie on live animals will become available. Would the application of such tests and the subsequent culling of all positive sheep, eventually lead to the eradication of scrapie and consist of an alternative for the introduction of scrapie resistant breeds?

Mathematical modelling has suggested that the best control measure is a test which will detect a scrapie infected sheep very early in the incubation period. How early this actually is could increase dramatically the power of the control programme. The tonsil or the third eyelid test for PrP^{Sc}, if these will work early enough in the incubation period could allow elimination of animals prior to lamb production. What is needed is to stop the transmission from affected animals to healthy animals, not necessarily to prevent disease in individuals themselves.

3.6. The theoretical possibility of BSE occurring in sheep: would the risks be similar to scrapie?

It is not certain that BSE has infected sheep. However within the UK, control measures should probably by now have ensured that there are at least no primary cases of BSE in sheep at present. One cannot exclude that BSE occurs in sheep in other countries throughout the world. There may be cross contamination from feeds which have meat –and-bone meal contribution. Good controls and supervision are both absolutely necessary to ensure the safety of sheep from BSE infection.

It is not certain that BSE will easily infect sheep. In the one published experimental study of oral infection of sheep with BSE, only one out of 3 fully susceptible animals actually succumbed to an oral dose of 0.5g BSE infected cow brain. In addition, *in vitro* conversion assay studies suggest that resistant genotype (ARR/ARR) sheep PrP^{C} protein is not converted to PrP^{Sc} by PrP^{BSE}.

The worst case scenario would therefore be that BSE has infected sheep and that it transmits within sheep in a manner similar to scrapie. Assessment of the risk of BSE in sheep depends crucially on whether BSE in sheep behaves like BSE in cattle (very probably is not horizontally transmitted) or scrapie in sheep (very likely to be horizontally transmitted). The pathogenesis of BSE in experimental sheep models has more resemblance to that of scrapie in sheep than it does to BSE in cattle. For example, BSE has been found in the spleen of experimentally challenged BSE affected sheep but not in BSE cattle spleen. Experiments are underway at various laboratories to study experimental sheep BSE in more detail. The effect of rendering on BSE infectivity would depend on the type of rendering plant in use. BSE might survive rendering procedures better than scrapie.

For reasons of human health protection, it will be politically less acceptable to have hidden, sub-clinical BSE infection in ARR/ARR resistant sheep – even though the amount of agent may be small - than it is to have hidden scrapie infection. However a resistant population would be expected to have very much lower titres of infection within them than would be found in susceptible sheep. A fully susceptible sheep population might show up any BSE present, but the background of infectivity in the preclinical, disease incubating sheep would be much higher and more dangerous. In addition at the moment, no biochemical test differentiates fully between scrapie and BSE although much work throughout Europe is aimed at developing such a test.

4. CONCLUSIONS

The Working Group concludes that the possibility that sheep may harbour a latent scrapie infection exists and if so, that they could pass an infection to other sheep. It is however expected that the background levels of infection in a resistant sheep population are significantly lower than in a susceptible population. There are firm indications that the scrapie agent, once introduced in an area, can persist in the environment and that maternal and horizontal transmission contributes significantly to the spread within a susceptible sheep population. Therefore the use of indicator animals or flocks only composed of sentinel susceptible sheep could increase the infective load in the environment and thus jeopardise the possibility to control and eventually eradicate scrapie. If BSE would have infected sheep, which is not certain today, and if BSE transmits and behaves in sheep in a manner similar to scrapie, for which there are some experimental indications, then a similar strategy for BSE and scrapie should be adopted.

The Working Group recommends the following steps as a preliminary to the introduction or steering in more E.U.-countries of scrapie-resistance breeding programmes:

- At the level of the EU: analysis and evaluation of the breeding programmes that are presently ongoing in a number of European countries (for example: France, The Netherlands, the United Kingdom, Norway, Sweden, …[4]); establishment of a register with the resistant breeds per country and their corresponding genotypes.
- At the level of each EU country: start the genotyping of large numbers of animals, in order to acquire a view on the distribution of the various genotypes in the national flocks. This would provide a first step towards the estimation of the distribution of genotypes involved in resistance against scrapie and, possibly, BSE.

The Group further recommends that, following the acquisition of these data, strong consideration should be given to the use of appropriate resistant strains of sheep, coupled with the development of and extensive use of validated diagnostic tests.

Before embarking on large scale breeding of sheep towards maximal resistant genotypes, according to the different breeds involved, consideration should be given to phenotypic characteristics.

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[4] see also the attached Working Group report

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ANNEX: NATURAL SCRAPIE IN SHEEP AND GOATS

Classification of tissues by agent titre in Swiss mice and by age, in pre-clinical and clinical cases of Scrapie in Suffolk sheep and in goats [5]

Group	Infectivity Titre (approx.range)	PRE-CLINICAL SHEEP ≤8 months.(0/16)	PRE-CLINICAL SHEEP 10-14 months(8/15) [6]	PRE-CLINICAL SHEEP 25 months(1/13)	PRE-CLINICAL SHEEP > 25 months(1/6)	CLINICAL SHEEP 34-57 months(9/9)	CLINICAL GOATS 38-49 months(3/3)
A	**HIGH** [3] **4.0**					Brain Spinal cord	Brain Spinal cord
B	**MEDIUM 3.2 – 4.0**		Colon-proximal, Ileum-distal, LN (RP/MP), Spleen	Colon-proximal, Ileum-distal, LN (RP/MP), Tonsil		Colon-proximal, Ileum-distal, Spleen, Tonsil LN (BM), LN (PF, 1/9 negative), LN (PS, 2/9 negative), LN (PR/MP), (rectum-distal+),	Colon-proximal, Ileum-proximal, LN (BM), LN (RP/MP), LN (s.mammary), Pituitary, (Rectum-distal +), Spleen
C	**LOW** £ **3.2 or titre unknown**		LN (PS/PF) Tonsil	Brain (medulla/ diencephalon), LN (BM), LN (PS/PF), Spleen		Adrenal, Bone marrow**, Colon-distal, CSF, Liver**, LN (s.mammary x2), Nasal mucosa, Pancreas **, Pituitary, Sciatic nerve, Thymus **,Placenta ** [6]	Adrenal, Colon-distal, CSF Nasal mucosa, Sciatic nerve, Thymus
D	**Undetectable**	Ileum, LN (PS/PF) LN (RP/MP), Thymus, Tonsil Spleen	Blood clot, brain (medulla), Colon-distal, Faeces, LN (BM), Serum	Adrenal, Brain (cortex mid-brain), Colon-distal, , LN (s. mammary), Nasal mucosa, Salivary glands, Spinal cord, Thymus	Colostrum	Blood clot, Fetus, Heart, Kidney, Lung, Mammary gland, Muscle-skeletal, Ovary, Saliva, Salivary gland, Sem. Vesicle, Testis, Thyroid, Uterus	Blood clot, Bone marrow, Faeces, Kidney, Mammary gland, Milk, Muscle-skeletal, Ovary, Salivary gland, Serum (see report), Uterus

(-/-) (Number positive / number examined)
* = Log_{10} mouse intracerebral LD/50 per 30 mg tissues
\+ = Not assayed but high content of lymphoreticular tissue
° = negative in other studies
** = trace or exceptional
PF = Prefemoral
PS = Prescapular
RP = Retropharyngeal
MP = Mesenteric/portal
CSF = Cerebro-spinalfluid
LN = Lymph node
BM = Bronchomediastinal

5 After Hadlow et al. (1979, 1980, 1982), Pattison *et al*. (1964, 1972), Groschup et al. (1996). Regarding DRG: see text.

6 Techniques for the determination of infectivity become more and more sensitive. The age range may go below 10 months. In individual cases, tonsil infectivity has been detected in lambs of 16 weeks. Placenta has been placed in Group C, but titres are unknown.