THE POSSIBLE VERTICAL TRANSMISSION OF BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

REPORT OF THE WORKING GROUP

SUBMITTED TO THE SCIENTIFIC STEERING COMMITTEE
AT ITS MEETING OF 18-19 MARCH 1999

I TERMS OF REFERENCE

In the light of the advice that milk is unlikely to be the route of infection in case of maternal transmission (for example: EC, 1996; SEAC, 1997, SEAC, 1998a,b), the following issues were addressed:

a) other possible routes of infection to explain maternal transmission of BSE

b) a risk assessment for these routes, and,

c) recommendations for options to mitigate the risk from these routes.

In this context the Working Group addressed the question: "*What is the nature and extent of the risks of vertical transmission (to include via semen, embryos or other ways of maternal transmission) of the BSE agent between cattle or between small ruminants of the same species, based on current data?"*

II CONTEXT OF THE QUESTION

In July 1997, the Commission requested the SSC to provide an opinion on maternal transmission (milk excluded), including a risk assessment and recommendation to mitigate the risk. In September 1997 the SSC provided a partial opinion covering only the safety of bovine semen and embryos. In January 1998 the Committee was requested to re-evaluate that opinion in the light of additional evidence that had since become available. A draft opinion was provided in June 1998 but did not take account of other possible routes of vertical transmission than *via* semen and embryos. Accordingly the SSC decided to merge these issues and to invite an Expert Working Group to make a report and provide a draft opinion for their consideration. A Working Group was set up. It met in Brussels in June, November and December 1998, twice in January 1999 and again in March 1999. The present document is its resultant report and conclusions.

III. ASSESSMENT

Preliminary remark:

Field data on the occurrence of BSE in countries that could potentially occur as a result of the import of semen or embryos from countries with BSE was not available to the Working Group and therefore could not be appraised. However, the Working Group is aware that information on imports of embryos and semen will be analysed by the Commission as part of the assessment of the BSE status of Member States and 3rd countries that submitted a dossier for their BSE status to be determined. The report hereafter may therefore be amended in view of the outcome of these analyses.

III.A GENETIC SUSCEPTIBILITY

III.A.1. Introduction

The *PrP* gene codes for the cellular form of prion protein PrP^{C} that can be post-translationally converted to the disease-specific form PrP^{Sc} following infection. This gene is responsible for the donor species effect (Kimberlin, Cole and Walker, 1989), that is one factor involved in the so-called species barrier. The species barrier is the

natural process that restricts or prevents the transmission of a TSE between species. It is operationally defined as the difference in incubation period (IP) between the first and second passages in the recipient species, *i.e.* IP of the second passage (within the recipient species) – IP of the first passage (donor species to recipient species). There is usually a significant reduction in IP at the second passage. The other factor in the species barrier is the strain of agent.

The donor species effect is related to the similarity of sequences of amino acids in PrP in the donor and recipient species. Of particular importance are differences in sequence at specific domains within the protein structure.

Even within a species and with a single strain of infecting agent there can be an apparent barrier to transmission between individuals. Sheep are a good example. In sheep, polymorphisms in the PrP protein are associated with the incidence of scrapie. Of particular importance are the amino acid codon numbers 136, 154, and 171 (Hunter and Cairns 1998). Specific allelic combinations are associated with differing incubation periods following controlled experimental challenge of sheep with TSE-infected brain material (Goldmann *et al,* 1991a, and Goldmann *et al,* 1994).

When scrapie is experimentally transmitted between sheep *via* a constant route and using a constant dose and strain of agent the outcome is dependent largely, if not entirely, upon the homology of the *PrP* gene sequence between the donor and recipient. The outcome could be no transmission (so-called scrapie-'resistant' sheep), transmission with a short incubation or transmission with a long incubation (both scrapie-'susceptible' sheep). Where transmission is unsuccessful it is not yet known whether a carrier state exists and if it does whether the individual excretes the scrapie agent such that it could be a source of infection for other sheep. In the case of non-transmission (*i.e.* clinical signs do not develop) this could be due to the incubation period being lengthened to beyond the natural lifespan of the individual. Furthermore, if a different strain of agent is used differences in incubation time can be reversed such that the sheep previously with a short incubation period have a long incubation and *vice versa.*

Polymorphisms in the goat *PrP* gene may also result in different incubation period lengths following experimental challenge depending on the amino acid sequence (Goldmann *et al*, 1996) but the situation is not as marked as in sheep. Completely scrapie-'resistant' goats have not been described. Polymorphisms in the cattle *PrP* gene have also been described but these do not correlate with disease occurrence (Goldmann, *et al* 1991b).

Naturally occurring mutations in the *PrP* gene, sufficient alone to cause disease, have not been described in animals. The known mutations are simply, in some cases, linked to disease susceptibility. If mutations, sufficient alone to cause disease do exist, they are assumed to be very rare indeed and phenotypically would present as familial diseases as they do in man. Confirmation of such a disease would require molecular genetic correlation of the mutation with disease occurrence in appropriate family members.

Somatic mutation in the *PrP* gene has been proposed in man as a hypothesis for the occurrence of sporadic CJD. However, such mutations have not been reported in any

case of sporadic CJD in man nor in any animal with a TSE. If they occurred they would not result in transmission down the germ line since gametes would not express the mutation. However, in sheep (the only species in which TSE is a proven contagious disease), it is hypothetically possible that 'sporadic' scrapie, if it occurred, could be transmitted in a similar way to conventional scrapie. In practical terms, scrapie and the other animal TSEs can be regarded as infectious diseases. The *PrP* gene can influence the incidence of disease, especially in sheep, in which species scrapie and possibly other TSEs such as BSE are contagious.

III.A.2. Definitions used in this document

Genetic susceptibility can be defined as the influence that different allelic combinations of the *PrP* gene (and possibly other as yet unidentified genes) can have on the outcome of exposure in some species or individuals, probably as a result of the effect on the period of incubation under defined conditions of agent dose, route of exposure and strain of agent.

Incubation period can be defined as that period between the date of exposure, or experimental challenge, and the date of onset of clinical signs. In experimental disease the incubation period can be accurately determined provided repeated and frequent clinical observations are carried out. In natural disease the date of exposure is usually not known and the date of onset of clinical signs may not be accurate, as clinical onset is insidious and may be determined retrospectively by the animal keeper. Thus the incubation period in individual cases of natural disease can rarely be accurately determined.

Vertical transmission is defined as:

- infection of the new-born, un-suckled offspring of infected or affected individuals in the absence of any other source of infection than that in the sire or dam or,
- The inheritance of genes that, independent of the presence of infection, will result in disease in all, or a proportion, of the offspring of the present or subsequent generation or,
- A combination of these factors.

Maternal transmission is defined as transmission of infection from the dam to the offspring *in-utero* or in the immediate post-natal period.

Embryos means embryos and ova.

PrP (a noun) stands for the prion protein. ***PrP*** (adjective) means the prion protein gene.

Low. In this document we use the term low in relation to risk, understanding the imprecision of the term. However, there are few data available to enable the Group to be more exact.

III.B. GENETIC SUSCEPTIBILITY IN SHEEP

III.B.1. Introduction

Genetic susceptibility of an individual is determined at conception and is lifelong. Whether or not disease results will depend upon environmental factors, principally contact with a TSE agent pathogenic for sheep *via* an appropriate route at sufficient dose to produce disease within the remaining lifespan. In other words susceptibility to a TSE does not mean disease will result. We do not know if the age at exposure is a factor that determines risk of disease developing. If a sheep is genetically susceptible to a TSE and is exposed, the dose, route of exposure and strain of agent will determine if disease results. The risk of exposure depends on the incidence of disease in the flock, the excretion of agent, the environmental contamination and husbandry factors. In regard to potential cumulative dose effects see Kimberlin (1979), Kimberlin and Walker (1989, 1990), and Diringer *et al,* (1998).

III.B.2. Genetic polymorphism of th *PrP* gene in sheep and links with susceptibility to scrapie agents

There are, to date, around 12 polymorphic amino acids in the sheep *PrP* gene. However studies of natural scrapie have confirmed the importance of three 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) and, although there are breed differences in PrP allele frequencies and in disease-associated alleles, some clear "rules" have emerged from this work. The most resistant genotype is AA136RR154RR171. Out of hundreds of scrapie affected sheep world wide, only one animal of this genotype has been reported with scrapie – a Japanese Suffolk sheep (Ikeda *et al*, 1995). This genotype is also resistant to experimental challenge with both scrapie and BSE (Goldmann *et al*, 1994). Other genotypes encoding QQ171 are more susceptible to scrapie. For example in Suffolk sheep the genotype AA136RR154QQ171 is most susceptible, although not all animals of this genotype succumb to disease and it is quite a common genotype amongst healthy animals (Westaway *et al*, 1994; Hunter *et al*, 1997). The PrP genetic variation in Suffolk sheep is much less than in some other breeds, the so-called "valine breeds". Breeds such as Cheviots, Swaledales and Shetlands encode *PrP* gene alleles with valine at codon 136 and the genotype VV136RR154QQ171 appears to be exquisitely susceptible to scrapie (Hunter *et al*, 1994; Hunter *et al*, 1996)]. VV136RR154QQ171 is a rare genotype and when it does occur, is almost always in scrapie-affected sheep and so it has been suggested that scrapie may be simply a genetic disease (Ridley and Baker, 1995). However, healthy animals of this genotype can live up to eight years of age, well past the usual age-at-death from scrapie (2-4 years) (Hunter *et al*, 1996; Hunter *et al*, 1997) and so the genetic disease hypothesis seems less likely than an etiology which involves host genetic control of susceptibility to an infecting agent.

In some "valine breed" sheep flocks affected by scrapie, there is survival advantage if genotypes encode certain PrP alleles, such as A136H154Q171 and A136R154R171 so that despite having a high-risk allele such as V136R154Q171, animals are unlikely to develop scrapie if their genotypes are VA136HR154QQ171 or VA136RR154RQ171 (Hunter *et al*, 1996). This has not been found to be the case in

all breeds or outbreaks however and recently genetic information has been summarised for many breeds in the UK (Dawson *et al*, 1998).

The answer could come from studies of experimental TSE in sheep where different sources of scrapie and BSE apparently target sheep according to their genotype at either codon 136 or codon 171. Following challenge by injection, the scrapie source SSBP/1 affects Cheviot sheep encoding the V136R154Q171 allele whereas the scrapie source CH1641 and BSE target sheep primarily according to codon 171 genotype producing disease with shortest incubation period in sheep which are QQ171 (Goldmann *et al*, 1994). With BSE valine at codon 136 seems to extend the incubation period. It is not known whether this applies also to CH 1641. Extending these findings to the naturally affected sheep, it is possible that there are also various types or strains of natural scrapie which target either particular sheep breeds and/or different PrP codons.

III.B.3. Susceptibility of sheep to BSE challenge

BSE when inoculated into NPU Cheviot sheep caused disease in animals of several different genotypes, with shortest incubation periods in AA136QQ171 genotypes (Goldmann *et al*, 1994). Codon 154 did not, in this experiment, have any influence on incubation period. This genetics makes BSE similar to CH1641 experimental scrapie, however the two TSEs are distinctly different. BSE transmits well to mice whereas CH1641 does not.

III.B.4. PrP genetics in goats

Analysis of the goat *PrP* gene revealed several different alleles and polymorphic codons, one of which (codon 142, isoleucine to methionine) resulted in an increased incubation period following experimental challenge with BSE, CH1641 scrapie and sheep-passaged ME7 scrapie (Goldmann *et al*, 1996). Recently a new caprine *PrP* gene allele has been reported and it contains only three instead of the usual five copies of a short peptide repeat and an additional tryptophan to glycine change at codon 102. The three-repeat allele is not, in heterozygous state, pathogenic, however may result in a longer incubation period following challenge with SSBP/1 scrapie (Goldmann *et al*, 1998). In contrast to sheep, at present, scrapie does not appear to be naturally sustainable in goats, although this possibility cannot be excluded.

III.B.5. Effect of route of infection

Using the intracerebral route and BSE infection, TSE was confirmed in 7 sheep out of 9 challenges (not counting intercurrent deaths). The oral route was less efficient with only 3 TSE cases occurring following challenge of 11 sheep with BSE. Two of the TSE cases in each group (ic and oral) may have actually been natural scrapie. The oral dose was 0.5g (of brain from cattle confirmed to have BSE) per sheep and likely BSE cases occurred with linkage to polymorphism of the PrP codon 171 genotype (Goldmann et al, 1994 and unpublished - additional TSE cases occurred in animals subsequently to the Goldmann et al, 1994 paper's publication).

Experimental infection of goats with BSE was more successful, 2 out of 3 challenged orally succumbed to disease as did 4 out of 5 challenged subcutaneously and 3 out of

3 challenged intracerebrally. Incubation periods depended on PrP genotype (Goldmann *et al*, 1996).

III.C. SUSCEPTIBILITY OF CATTLE

III.C.1. Genetic polymorphisms in cattle

Polymorphisms in the bovine *PrP* gene have been described in British cattle (Goldmann *et al* 1991, Martin *et al* 1991,) and confirmed by Yoshimoto *et al* (1992), and Prusiner *et al* (1993). Polymorphisms in the bovine *PrP* gene have also been described in Belgian cattle (Grobet *et al* 1994) and in US cattle (McKenzie *et al* 1992; Ryan and Womack 1993, and Robinson *et al* 1995). There are two polymorphisms in the coding region of the bovine *PrP* gene. One is a HindII restriction site polymorphism. The other is a difference in the number (5 or 6 copies) of an octapeptide repeat sequence (Goldmann *et al*, 1991, Hunter *et al*, 1994). An analysis of data on the *PrP* genotype and the occurrence of BSE revealed no difference in the frequency of these genotypes in 370 cattle in Scotland with and without natural BSE. A similar result was found in the study by Robinson *et al*, (1995) in the USA. In both studies the 6:6 genotype was most frequent, the 6:5 genotype was moderately frequent and the 5:5 genotype was rare (UK) or absent (US). The UK authors suggested that the 6 octapeptide allele could be dominant for susceptibility to BSE. However, they also noted the infrequency of occurrence of BSE in the cattle population at large (ϵ 1% incidence in the adult population at the peak of the epidemic) in which the 6:6 allele dominated.

Deficits in knowledge:

It is not known if flanking regions of the *PrP* gene promoters or other genes than the *PrP* gene regulate BSE occurrence.

III.C.2. Susceptibility to the BSE agent

a) experimental evidence

All cattle that have been challenged either orally or parenterally with the BSE agent in brain and have lived out the incubation period have succumbed to disease[1]. Of particular note is the uniform susceptibility and incubation period in two breeds of genetically unselected cattle when challenged intracerebrally and intravenously with brain material from confirmed BSE cases (Dawson *et al* 1991, 1994; Kimberlin and Wilesmith, 1994). In this study the dose, route of infection and strain of agent was controlled but genetic variation was not. This contrasts markedly with the results one would expect if scrapie agent had been inoculated into a genetically diverse population of sheep.

b) epidemiological evidence

[1] Regarding oral challenge, various doses have been used: see for example Dawson *et al* (1991, 1994), Wells *et al* (1998)

There is a low average incidence of BSE in affected herds. For example, 34.8% of farms have had only one case to 1 November 1998 and 82% have had 7 cases or less (MAFF, Personal Communication 1998). Most cases have occurred in the Holstein Friesian breed. This is not because of genetic predisposition but rather reflects the relative numerical size of the breed compared with other dairy breeds. Thus within dairy breeds the incidence of BSE is similar (Bradley and Wilesmith 1993).

Since there is no basis for implicating genetic susceptibility in the cause of BSE in the general epidemic other explanations have to be considered. A more plausible explanation for the low incidence of BSE is that the average exposure to infection in feed was at a low level. Furthermore effective doses of infectivity probably occurred in packets that were randomly distributed within feed batches. The concentration of packets could vary between batches and over time and would determine the risk factor for an individual bovine animal consuming the feed.

A cohort study to examine maternally-associated risk factors for BSE (Wilesmith *et al* 1997) concluded that there was a significantly enhanced risk of BSE among offspring of BSE-affected dams compared with their matched controls. We cannot exclude the possibility that some form of enhanced genetic susceptibility contributed to this risk enhancement.

In the absence of data on the *PrP* gene in these animals, variations in this gene cannot be excluded.

c) Conclusion

It is concluded that on current evidence, it is most likely that all cattle are susceptible to BSE. The role of genetics in BSE appears to be minor and especially in regard to polymorphisms in the bovine *PrP* gene. The role of genetics in the susceptibility of cattle to low dose infectivity in feed or in connection with maternal risks for the offspring is hypothetical and has currently no molecular basis.

III.C.3. Susceptibility to scrapie agents

a) experimental evidence

US cattle of different breeds are partially susceptible to passaged or naturally occurring strains of scrapie from US sheep and goats but only by routes that include the intracerebral route (Hourrigan, 1990; Gibbs *et al,* 1990; Cutlip *et al,* 1994; Clark *et al,* 1995,). Interestingly, Cutlip *et al,* (1997), reported the results of a second passage in cattle by the intra-cerebral route in which three of four inoculated calves succumbed to a neurological disease after a similar incubation period as in the first study. One calf died from bloat and was excluded. These results suggest that the species barrier between sheep and cattle may be small and that a genetic influence is undetectable. The disease produced in the various studies showed a variable attack rate and clinical neurological signs that were distinguishable from those observed in cattle with BSE in Europe. There was also minimal morphological pathology in the brain but PrP^{Sc} was consistently detected

in the brain of cattle with clinical signs. No molecular genetic studies have been reported.

b) epidemiological evidence

No strain of scrapie agent has been isolated from natural cases of TSE in cattle.

c) Deficits in knowledge

It is not known if the strains of agent present in natural cases of sheep and goat scrapie in Europe are pathogenic for cattle. Experiments to investigate this are underway. It is also not known for certain that any genetic rules that apply to experimental parenteral challenge, apply in the same way to oral challenge

d) Conclusion

The occurrence and mechanism of genetic susceptibility of cattle to experimental scrapie is unknown but current evidence suggests it may be similar to that in experimental BSE.

Natural scrapie infection in cattle is a hypothetical concept (Diringer, 1995) and has not been reported anywhere in the world. All isolates of TSE agents from cattle with TSE have had the characteristic strain type profile of the BSE agent which is quite distinct and separate from the profiles of known scrapie agents (Bruce 1996).

III.D. DISTRIBUTION OF TSE AGENTS WITHIN THE HOST IN REGARD TO THE REPRODUCTIVE ORGANS

III.D.1. Introduction

Infectivity can only be detected by bioassay. Detection of PrP^{Sc} is often used as a proxy for infectivity in the practical sense but it is already known that not all such PrP is infectious (Somerville & Dunn, 1996) so caution must be exercised. In any event the detection of PrP^{Sc} even by the most recently developed methods is about 1000 times less sensitive than bioassay.

Bioassays are most sensitive when conducted within the same species and taking account (in sheep for example) of polymorphisms in *PrP* gene of the donor and recipient. For reasons of consistency, practicality, speed and cost most bioassays are conducted in laboratory animals. However, this results in a loss of sensitivity. For example, in an incomplete comparative bioassay of BSE infectivity from natural cases of BSE conducted in mice and in cattle, the results suggest that the former species was at least 1000 less sensitive than the latter (Wells *et al* 1998). The calculated limit of detection in mice is about 25 mouse i/c LD_{50}/g (but 25.000 cattle i/c LD_{50}/g: see footnote to **TABLE 2)** for 20µl i/c and 100 µl i/p doses of a 10% homogenate. (See also: Fraser *et al* (1992), Foster *et al* (1996) and Diringer (1999)).

Despite these draw backs it is possible to determine the titres of agent in different tissues and compare them with those in highly infected tissues like brain from clinical cases. This enables a judgement to be made on the relative risk from different tissue.

It is stressed that absence of detectable infectivity in the tissue does not mean that infectivity is absent. It might be, but alternatively infectivity could be present. (See also the footnote to **TABLE 2**). It is then a question of judgement as to whether such a level of infectivity is an acceptable risk taking account of all the other factors involved. These factors, in the context of semen and embryos, include any processing that is done before use.

III.D.2. Results of bioassay of TSE agents

a) Scrapie and BSE in small ruminants

Extensive studies of tissue infectivity during the incubation period and in the clinical phase of disease of natural scrapie in Suffolk sheep have been reported by Hadlow *et al* (1982). Hadlow *et al* (1980) reported similarly in regard to tissues from goats affected with clinical scrapie. The results are summarised in **TABLE 1**.

No reproductive tissue from male or female animals showed detectable infectivity. However, the bioassays were done in mice so it is only possible to say that the infectivity in these tissues was of the order of 1000 times lower than that in brain. Few (sheep) or no goat studies were conducted in the pre-clinical phase of disease.

Hourrigan (1990), reported the outcome of tissue transmission studies conducted in association with Dr Marguerite de Camp in the same US flock as studied by Hadlow *et al* (1982). In regard to male and female reproductive tissues, milk and colostrum bioassayed in mice no detectable infectivity was found in testes (0/6), semen (0/21),

TABLE 1: 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 [2]

Group	Infectivity Titre (approx.range)	PRE-CLINICAL				CLINICAL	
		SHEEP				SHEEP	GOATS
		≤8 months.(0/16)	10-14 months(8/15) [3]	25 months(1/13)	> 25 months(1/6)	34-57 months(9/9)	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 **	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)
** = trace or exceptional
MP = Mesenteric/portal
* = Log_{10} mouse intracerebral LD/50 per 30 mg tissues
PF = Prefemoral
CSF = Cerebrospinal fluid
+ = Not assayed but high content of lymphoreticular tissue
PS = Prescapular
LN = Lymph node
° = negative in other studies
RP = Retropharyngeal

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

[3] 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.

BM = Bronchomediastinal

milk (0/6) or colostrum (0/3).[4] It is noted that Palmer (1959) also failed to detect infectivity in semen from a ram with confirmed scrapie following bioassay in lambs. Continuing with Hourrigan's report, infectivity was found in 4/14 ovaries, 4/13 uteri, 1/10 uterine caruncles, 1/1 samples of amniotic fluid, 2/10 fetal placental cotyledons and 1/13 fetuses. This contrasts with the negative results in the earlier study of the sheep, the route of inoculation, the attack rate, length of the incubation period and titre in the mice and the method of judging that scrapie had occurred. Unfortunately no detail is given in Hourrigan's report such as the stage of incubation of the sheep, the route of transmission, the attack rate, length of the incubation period and titre in the mice and the method of judging that scrapie had occurred. It is therefore unwise to place too much credence on these data until the study is repeated with similar results. However, some of the data are in line with other reports such as infectivity in the placenta of sheep as reported by Pattison *et al* (1972 and 1974). In this study placenta from scrapie affected Swaledale sheep transmitted the disease to both sheep and to goats by the intracerebral route and by the oral route. Onodera *et al* (1993) reported finding infectivity in the placenta of sheep in Japan with natural scrapie following bioassay in mice. They did not find PrP^{Sc} in the placenta.

However, Race, Jenny and Sutton (1998), reported finding PrP^{Sc} in the placenta of scrapie-infected sheep and infectivity in the placenta was also confirmed. Interestingly in two sheep PrP^{Sc} and infectivity was found in the placenta following one pregnancy but not in that at the next pregnancy, when one of the sheep had clinical scrapie. This has yet to be explained. No information on the PrP genotype of the placentae was given.

It is noted that the titre of infectivity in ovine placenta has not been reported. The role of placenta from goats with scrapie is also a deficit in knowledge. Infectivity in reproductive tissues of experimentally scrapie-infected or BSE-infected sheep has not been reported but some studies are in progress. BSE as a natural disease of sheep has not been reported either.

Infectivity studies on ovine and caprine milk and colostrum by intracerebral inoculation of homologous species are not available.

b) BSE in cattle

Tissue infectivity studies

Tissue infectivity studies in cattle clinically-affected with natural BSE have revealed no detectable infectivity in any of the following tissues bioassayed in mice (**TABLE 2** and see MAFF (1998), with acknowledgements to Middleton and Barlow (1993), Fraser (1994) and Taylor *et al* (1996)): milk, ovary, embryos, placental cotyledon,

[4] **Note**: as with the bioassay of tissues from cattle with BSE – see also the footnote to Table 2 **–** these bioassays were conducted across a species barrier and therefore were likely to be less sensitive than conducting them within species and taking account of PrP polymorphisms in sheep.

amniotic and allantoic fluids, uterine caruncle, mammary gland, epididymis, prostate, semen, seminal vesicle and testis (see also the footnote to **TABLE 2**). Cattle have been challenged oro-nasally with placental tissue from cattle affected with BSE late in gestation, but no evidence of disease was found during the course of incubation or at seven years after challenge when the recipients were killed whilst healthy. Embryos derived from clinically BSE-affected cattle and resulting from mating by AI with semen from either healthy bulls or bulls with confirmed BSE have been transferred, using IETS protocols, into 347 BSE unaffected cattle imported from New Zealand. No case of BSE has developed in either the recipient cows or the offspring, some of which have now been killed having reached the 7 years termination point (data courtesy of Dr A E Wrathall). The experiment will be completed in 2001. See Paragraph III.I.2 and III.J.2 for details.

Infectivity studies in reproductive tissues of cattle during the incubation period in a pathogenesis study of experimental BSE have not been reported. The cattle in this study were all castrated males. New studies are in progress.

Deficits in knowledge:

Infectivity studies on bovine milk, colostrum, placenta, semen and embryos by intracerebral inoculation of cattle are not available.

c) Scrapie in cattle

Scrapie is not known to occur as a natural disease in cattle. In the experimental studies of scrapie in cattle conducted in the USA no reproductive tissue bioassays have been reported. Some studies of the experimental disease in cattle are in progress in the UK.

III.E. TRANSMISSION DURING GESTATION

III.E.1. Transmission of scrapie agents in sheep

It is part of the dogma of scrapie that the disease in sheep is maternally transmitted to the lamb, with the placenta as prime suspect as the vehicle both *in utero and post-natally*. Telling the difference between genuine maternal transmission and lateral transmission by close contact during the perinatal period is difficult and it is not always possible to know when an animal has acquired its infection. However in a natural scrapie outbreak in the NPU Cheviot flock (Hunter *et al*, 1996 and unpublished), it seems likely that most animals were infected at about the same age. The ages-at-death from scrapie were constant with VV136RR154QQ171 animals dying at 700-900 days of age and heterozygotes VA136RR154QQ171 dying at 1100-1200 days of age. This difference between homozygotes and heterozygotes, expected from the experimental data, is not always seen in other flocks (Hunter et al, 1994).

TABLE 2: Natural BSE-tissues with no detectable infectivity mouse challenge by parenteral (I/C I/P) inoculation[5]

SPLEEN LYMPH NODES Mesenteric Prefemoral Retropharyngeal	SKELETAL MUSCLE M. Diaphragma M. Masseter M. Longissimus	LIVER KIDNEY PANCREAS	TESTIS EPIDIDYMIS SEMINAL VESICLE PROSTATE SEMEN	OVARY UTERINE CARUNCLE PLACENTAL COTYLEDON AMNIOTIC FLUID ALLANTOIC FLUID
TONSIL	M. Semitendinosus			
				EMBRYOS
	BONE MARROW BUFFY COAT SERUM BLOOD CLOT FETALCALF BLOOD	OESOPHAGUS RETICULUM RUMEN Oesophageal Groove Pillar		
CAUDA EQUINA PERIPHERAL	 MAMMARY GLAND MILK HEART	OMASUM ABOMASUM		
NERVES N. Sciaticus N. Tibialis N. Splanchnicus N. Optic		SMALL INTESTINE Proximal & Distal COLON Proximal & Distal RECTUM		
CEREBRO-SPINAL FLUID	MIDRUM FAT SKIN			
		LUNG TRACHEA		(DEC 1998)

However, the survival of three VV136RR154QQ171 animals to over 1900 days in this flock after stringently clean procedures were used around the time of their birth suggests that the perinatal period is a dangerous time for a susceptible lamb. This period is now being further investigated by comparing caesarian-derived and bottle-fed lambs with lambs born naturally and suckled by their dam.

Embryo transfer (ET) procedures (Stringfellow and Seidel, 1990) have also been investigated in the hope of bypassing any infection cycle. Although work with the NPU Cheviot flock was compromised by natural scrapie in the flock (Foster et al, 1992;) it is clear that ET procedures coupled with stringent control of cleanliness at

[5] The limit of detection of infectivity by i/c inoculation (the most sensitive route) of mice with cattle tissue is approximately 25.000 i/c cattle ID50 per gram of a 10% brain homogenate.

birth, do not prevent scrapie from occurring in all lambs. These experiments generated the three surviving VV136RR154QQ171 animals discussed above, but also generated 10 VV136RR154QQ171 sheep which did develop scrapie (9 at mean age 826 + 24 days of age and one at 1267 days of age). This group of 10 animals was not protected by ET procedures even after following International Embryo Transfer Society (IETS) protocols (Stringfellow and Seidel, 1990). A similar experiment set up in the USA (Foote *et al*, 1993) is difficult to interpret because of lack of PrP genotype information. However one of the groups involved Cheviot sheep inoculated with SSBP/1 scrapie and it has already been shown that these animals give similar incubation periods to NPU Cheviots and SSBP/1 (Maciulis *et al*, 1992). The incubation periods in scrapie-inoculated donors and recipients ranging from 6.1m (183d) to 14.9m (447d), plus survivors to 25m (750d), suggests that a mixture of *PrP* genotypes is present in the group. Of the positive control lambs (gestated and born naturally) 2 out of 9 developed scrapie at 31m (930d) and 49m (1470d) of age whereas none of the 22 ET derived Cheviot lambs succumbed to scrapie with ages at death ranging from 74.5m (2235d) to 96m (2880d). It is of course possible that there were no susceptible lambs amongst the ET group. But there is at least a suggestion that ET procedures that involved three, as distinct from ten embryo washings recommended by the IETS, may have protected lambs from scrapie in this case and again implicating the possibility of maternal transmission as a route for scrapie transmission. Further experiments are being carried out to clarify the situation.

III.E.2. Transmission of BSE in sheep and goats

Nothing is known as yet about maternal transmission of BSE in sheep. Hunter and Foster (Institute for Animal Health, Neuropathogenesis Unit, UK) have a MAFF funded project underway designed to look at the possibility of maternal transmission of BSE in sheep but no results are available at this time.

In a study of maternal transmission of BSE in goats, embryos were transferred from BSE-infected donor mothers at 6 days of gestation into uninfected recipients and allowed to be born naturally. The donors had been challenged with BSE about 390 days prior to natural mating and all subsequently succumbed to BSE with incubation periods linked to codon 142 of the *PrP* gene (Goldmann et al, 1998). There were 37 live offspring, 22 of which survived until culling at over 2000 days of age. There were no cases of a TSE-like disease in any of the offspring or in the male goats which fathered them. This was confirmed by immunocytochemistry and Western blotting for PrP^{Sc} in brain tissue sections and extracts of brain respectively. This study would have detected a 5% vertical transmission rate if the infection had passed from the inoculated donor mother into her germ line during the 400 days prior to the transfer of her 6 day-old embryos. The study does not address the question of whether infection can pass to the embryo at later stages of gestation in the surrogate dam (it is not possible to transfer embryos much later than 6 days old) nor whether, kids born naturally to infected mothers at times very close to onset of clinical signs would have become infected. (Foster *et al*, Journal of General Virology, in press).

III.E.3. Transmission of BSE in cattle

a) Experimental evidence

There is no evidence for the transmission of BSE during gestation as a result of embryo transfer (though it is noted that the current study in cattle is advanced but as yet incomplete). Please see Sections III.D and III.J.

b) Field evidence

There is evidence from a cohort study for low level maternal transmission. See Section III.C2 b).

III.E.4 Transmission of scrapie in cattle

No results have been reported on studies of experimental scrapie in pregnant cattle. Scrapie has not been reported as a natural disease in cattle.

III.F. OTHER PARENTERAL INFLUENCES

III.F.1. Genetics

With our current knowledge of the role of the *PrP* gene in influencing the incubation period of scrapie in sheep and goats it is clear that the sire and dam contribute to vertical transmission of this trait. The role of the *PrP* gene in other domestic animal species is incompletely known.

III.F.2. Infection

Even though the nature of TSE agents is not yet fully understood, there is stronger evidence that the ewe is the major, or possibly even the only, source of infection for the offspring or other contact sheep or goats. Evidence to support this view is given below.

Experimental studies with scrapie-infected sheep placenta show the disease can be transmitted to sheep and goats by the oral route (Pattison *et al*, 1972, 1974), thus suggesting a mechanism for horizontal transmission in the field.

Furthermore, other studies by Pattison (1964) showed that "contact spread of scrapie has never been observed during 18 years of experimentation with scrapie in sheep and goats where only rams or non-pregnant lambs or ewes, where used as scrapie-affected donors."

Transmission to the fetus during pregnancy cannot be excluded. However, Hadlow *et al* (1984) inoculated seven new-born lambs and 21 fetal lambs *in utero* with scrapie. No scrapie infectivity was found in lymphoreticular tissues from the new-born lambs up to 147-210 days later. In the fetuses no infectivity was detected up to 254 days after birth. Traces of infection were found in 2 lambs at 254 days and in one lamb at 322 days of age but only in peripheral and mesenteric lymph nodes. Inability to detect infection could have been due to a lack of sensitivity of the mouse bioassay, the long zero phase or the lack of replication. A similar experiment using twin goat fetuses was described by Pattison (1964). When both were inoculated they each succumbed to scrapie 9 months after inoculation, 7 months after birth. In the other

only one twin was inoculated and only it developed scrapie 7.5 months after inoculation, 5.5 months after birth**.**

Preference for maternal over paternal transmission in offspring studies was recognised by Matthew***s*** (1967) and has been reported by Dickinson, Stamp and Renwick (1965) and Hourrigan *et al* (1979). There are two possibilities to explain this observation:

- Infection during gestation (direct vertical transmission)
- Infection post-natally that could include infection from placenta, colostrum and milk (indirect vertical transmission).

It is well known that sometimes scrapie incidence in a flock increases some years following the introduction of a ram, for example see Ducrot and Calavas, (1998). Whereas in theory this could be associated with introduction of infection, a more likely explanation is that the new ram introduces *PrP* gene alleles associated with short incubation period and increased susceptibility. This could elevate the incidence of a previously-existing infection that was resisted by the majority of breeding sheep.

There are no clear studies that prove or disprove these routes except for placenta under experimental conditions. Further studies (some in progress) are needed to understand the mechanism of maternal transmission in ruminants and particularly in sheep experimentally infected with scrapie and BSE.

Epidemiological studies on bovine milk

In regard to milk being a risk of causing maternal transmission the following points should be noted. First the greatest risk would be for cattle as there is no species barrier and calves could consume vast quantities if suckled by their BSE-infected dam. Such a situation arises in the UK, beef suckler herd.

Offspring of BSE-Affected Pedigree Suckler Cows

Preliminary results from an on-going study of the offspring of BSE-affected pedigree suckler cows which had been suckled for at least one month was reported by Wilesmith & Ryan (1997). 126 homebred animals that had developed BSE by August 1996 were identified in 90 pedigree beef suckler herds. These animals had produced 234 offspring, of which 219 had been suckled by their own dam for at least 1 month. Of these, 132 offspring had survived to at least 20 months of age, the youngest recorded age at onset of clinical signs of BSE in Great Britain. There were no cases of BSE recorded in these animals. Further analysis show that these data are consistent at the 95 % level with a rate of direct maternal transmission of 0-17.3 % in the last 11 months of the dam incubation period, or 0-8 % in the last 23 months of the dam incubation period (Donnelly 1998). The results of this study could not discriminate between tissue sources of infection such as, for example, placenta, milk or colostrum.

The second point to note is that dairy calves, like beef calves, receive colostrum but there is a vast difference in incidence in favour of dairy cattle in the occurrence of BSE. This is explained by the different feeding of dairy calves and inclusion of MBM in their diet before bans were in place.

As regards the risks from bovine milk, the Working Group refers to the continuous review of the UK Spongiform Encephalopathy Advisory Committee SEAC's. SEAC has regularly discussed the safety of bovine milk in regard to BSE, the last time on 9 November 1998. The latest substantive SEAC view, expressed on 16 April 1997, was that the measures currently in place to protect the consumer were considered appropriate. (UK law states that milk derived from BSE affected cattle or cattle suspected to have BSE shall not be sold, supplied or used for human or animal consumption, with the exception that it may be fed to the cow's own calf.) SEAC concluded then (16/4/97) that no evidence had been found to suggest that milk from any species affected by transmissible spongiform encephalopathies was infectious. The Committee is keeping the possible risk infectivity in milk under review and stated most recently on 14 May 1998 that there was no reason to change their previous advice on the safety of milk. This advice may need to be updated as new data and information become available.

III.G. HORIZONTAL TRANSMISSION THROUGH PARTS OF THE CONCEPTUS EXCLUDING THE FETUS ITSELF

III.G.1. Scrapie in small ruminants

Once scrapie has been introduced to a flock or country, more often than not the disease becomes endemic, though draconian control methods, including flock destruction, may reduce, but rarely eliminate the problem. There is good evidence from several sources (see Section III.D above) that the placenta and perhaps fetal fluids contain infectivity and that scrapie-infected sheep placenta can experimentally transmit the disease by the oral route. There is also evidence that environmental contamination with the scrapie agent such as is likely to occur when breeding sheep in a scrapie-infected flock are housed and in close contact, especially at lambing time, can lead to further extension of scrapie to unrelated susceptible members of the flock. For further discussion on indirect transmission see Brotherston *et al* (1968), Pattison (1964), Sigurdarson, (1990) and Rubenstein *et al* (1998).

III.G.2. BSE in small ruminants

BSE has not been reported as a natural disease of small ruminants. No studies of experimental BSE in pregnant sheep have been reported but such studies are in progress and will potentially provide the opportunity to investigate horizontal transmission of the experimental disease and particularly the role of the placenta and its fluids in transmission or potential for transmission.

III.G.3. BSE in cattle

None of the reproductive tissues or any parts of the conceptus that have been tested have shown detectable infectivity (**TABLE 2** and footnote to this table). Studies on the enhanced risk of BSE in calves born to BSE-affected dams are discussed in Section **III.H**. These studies are unable to reveal a mechanism for maternal transmission and therefore unable to distinguish between transmission during gestation and transmission through parts of the conceptus excluding the foetus itself during or following birth of the calf.

III.G.4. Scrapie in cattle

No studies of experimental scrapie in pregnant cattle have been reported. Scrapie agents have not been reported from natural cases of TSE in cattle.

III.H. ENHANCED RISK OF BSE IN THE OFFSPRING OF DAMS WITH BSE

None of the reproductive tissues or any part of the conceptus that has been tested has shown detectable infectivity (see **Paragraph D**). Several studies have been undertaken to examine maternally-associated risk factors for BSE (see below). However, none of these studies reveal a mechanism for maternal transmission.

III.H.1. Scientific Background

a) Cohort Study to examine maternally-associated risk factors

The cohort study was initiated in July 1989 by the Central Veterinary Laboratory in Weybridge, UK, to assess maternally-associated risk factors for BSE (Wilesmith *et al.* 1997). The study was designed as a matched control study. The incidence of BSE in the offspring of cows that developed clinical signs of BSE[6] is compared with the incidence of BSE in offspring, born in the same season and herd, of cows that had reached at least six years of age and had not developed BSE (the controls). Animals in the exposed arm of the study were recruited from the offspring of dams identified in the BSE database. Individuals in an exposed control pair were matched by natal herd and birth date to minimise differences between them. Pairs were subsequently excluded from the study if either animal died before March 1 1990 or if the dam of the control animal developed BSE at any time. The study was unpredictably confounded by the continued, but progressively reducing risk of BSE-infection from a feed-borne source, due to imperfections in the ruminant protein feed ban that became evident later. In this context it is noted that there is some evidence of a period effect, in that risk differences between the two cohorts reduced, the later the animals were born. However, these differences were not statistically significant because of the limited number of cases of BSE and the small number of cattle in the study that were born before the feed ban was introduced.

301 pairs of animals were followed, that were recruited from 268 natal herds. The BSE-status of the animals is summarised in the **TABLE 3** below.

TABLE 3 NUMBER OF OFFSPRING WITH AND WITHOUT PATHOLOGICALLY CONFIRMED BSE IN THE EXPOSED ARM AND CONTROL ARM.

		Exposed	
		+	-
Controls	+	6	7
	-	36	252

[6] This offspring is referred to as “exposed” in the remaining part of Section III.H.1.

The data show a significantly enhanced risk of BSE among the offspring of affected dams compared with their matched controls ($p<0.0001$). This enhanced risk may have arisen from direct maternal transmission of the aetiological agent of BSE, in which case the probability of maternal transmission is estimated as 9.6% (95% confidence interval 5.1% - 14.2%). Alternatively, this enhanced risk could be due to enhanced genetic susceptibility in the exposed arm, where the estimated relative risk, 3.23 (95% CI 1.77 - 5.89), is an indicator of the level of heterogeneity of susceptibility (which can then be related to the relative susceptibilities of the underlying genotype (Ferguson *et al.* 1997)). The study concluded that the results indicated an increased, but low, maternally-associated risk in the offspring of BSE cases. It could not distinguish between an unidentified genetic component and true maternal transmission or a combination of each**.**

It is planned to conduct a blind study of the *PrP* genetics in some affected and unaffected cattle in the cohort study by recovery of DNA from paraffin blocks of brain tissue. This study is to be carried out by the Institute for Animal Health in the UK. Other collaborative genetic studies in cattle may also shed light on the role of genes in the susceptibility of cattle to BSE.

Three independent statistical analyses of the data have been undertaken (Curnow *et al.* 1997, Donnelly *et al*. 1997a, Gore *et al.* 1997). A key indicator of direct maternal transmission in these data is the variation of the maternally enhanced risk over the dam incubation period, with a greater enhanced risk in animals born close to and following the onset of clinical signs in the dam. All three analyses concluded that the data support significant direct maternal transmission at a rate of approximately 8-10% over the last 150 days of the maternal incubation period. However, the analyses were not able to exclude additional enhanced genetic susceptibility in the absence of detailed data on the genotypes of individual animals.

b) Analysis of Dam-Calf Pairs of BSE Cases

i) Cases born prior to the introduction of the ruminant feed ban in 1988 in Great Britain.

Bradley and Wilesmith, (1993) report a comparison of the observed annual incidence of BSE in the offspring of confirmed cases in Great Britain and the expected incidence from the feed-borne sources for onset years 1988/89, 1989/90, 1990/91, 1991/92 and 1992/93. They show that the actual incidence is always less than that expected from feed-borne sources. These results could be consistent with low levels of maternal transmission.

ii) Cases born after the introduction of the ruminant feed ban in 1988 in Great Britain.

Further information on a maternal risk enhancement is contained within the large database of BSE case reports in Great Britain, in which the dams of animals born following the introduction of the ban on the use of ruminant material in cattle feed in July 1988 were traced. A detailed analysis of these data was undertaken by Donnelly *et al.* (1997b). The analysis was based on the determination of the BSE status of 31,192 confirmed cases born after the

introduction of the ruminant feed ban ('Born After the Ban' cases or BABs). A high proportion, (85.9%), of dams of BAB cases were identified. 1346 BSE-affected dam-calf pairs were identified in the database.

Assuming that dams calve annually - and using data recording whether the animal was pregnant and, if so, the stage of pregnancy - an estimate can be made of the expected number of dam-calf positive pairs that would occur by chance. The ratio of the observed to expected numbers was calculated by dam incubation stage. The results showed a significantly enhanced risk in offspring born to BSE-affected dams close to or following the onset of clinical signs in the dam.

As in the maternal cohort study, this maternal risk enhancement could be due to direct maternal transmission of the aetiological agent, enhanced genetic susceptibility, or some combination of these two factors. However, the variation in risk enhancement over the incubation period of the dam can only be explained by direct maternal transmission. Further statistical analysis of the data gave estimates of significant direct maternal transmission in the last 24 months of the dam incubation period, with highest rates (8 - 12%) in animals born after the onset of BSE in the dam.

iii) Offspring of BSE-affected animals in Switzerland.

A study of the offspring of BSE-affected dams was reported by Braun *et al* (1998) and Fatzer *et al* (1998). 182 offspring of BSE-affected dams were followed. Only 8 of the offspring were born prior to the end of 1990 when a ban on the use of MBM for ruminants came into place. None of the offspring showed clinical signs of disease or were diagnosed as BSE cases on histological and immunohistochemical examination of the brain. However, over half of the animals did not survive for a sufficient length of time to ensure that BSE could be detected in an infected animal.

Eight animals born to BSE-affected dams within 6 months of clinical onset survived a sufficient length of time to detect BSE, whilst there were 17 such animals born to BSE-affected dams within 12 months of clinical onset. Assuming that none of these animals was infected, these results give 95% confidence intervals for direct maternal transmission of (0% -31%) in the last 6 months of the dam incubation period and (0% -16.2%) in the last 12 months of the dam incubation period.

c) Case-control study of animals born after the ruminant feed ban

Hoinville *et al.* (1995) undertook a case-control study in 1994 of BSE cases born after the ruminant feed ban to examine the potential roles of maternal and/or horizontal transmission. The offspring of cattle that subsequently succumbed to BSE were not found significantly more often among the cases and hence the results of this study did not indicate any statistically significant vertical transmission of BSE. Other studies confirmed that compliance with and enforcement of the feed ban had been imperfect (but improving with time) and that

exposure of cattle born after the date of the ban could have been exposed to infection in feed by cross-contamination of concentrate feed in mills (Wilesmith 1996). However, because of the sample size, the results were consistent with rates of direct maternal transmission between 0 and 13%.

d) Other data

Of the approximately 1,000 cases of BSE that have occurred in native-born cattle outside the UK, none has been reported in the offspring of BSE cases.

III.H.2. Conclusion

There is an enhanced risk of approximately 10% of BSE in offspring born to BSE-affected dams. The results of all epidemiological studies undertaken to date have been consistent with a rate of direct maternal transmission of approximately 10%, in calves born to dams within 12 months of onset of clinical signs of BSE, with lower rates up to 24 months prior to the onset of clinical signs in the dam. Enhanced genetic susceptibility cannot be excluded on the basis of these data but such genetic susceptibility at present is only speculative. On the basis of these data the UK SEAC concluded that there is some evidence of direct maternal transmission at a low level but they cannot rule out variation in genetic susceptibility to feed-borne infection as an additional factor. It is thus still unclear if maternal transmission of BSE in cattle in the traditional sense occurs or not and if it does the mechanism involved. Even if it does occur the epidemic in the UK will not be maintained.

Remark:

There is even less evidence for horizontal transmission. If horizontal transmission occurs as a rare event there is no knowledge of the source of infection that could cause it.

III.H.3. Areas for Future Research

- Continued analysis of the offspring of BSE-affected dams in the BSE case database in Great Britain, coupled with molecular genetic analysis/population genetic studies, may refine estimates of direct maternal transmission and enhanced genetic susceptibility.
- Further updates on the offspring of pedigree beef-suckler cows may be useful to refine rates of direct maternal transmission and to assess the risk of direct maternal transmission through milk compared to other routes.

III.I. RISKS LINKED TO SEMEN

III.I.1. Introduction

a) Natural mating

There are no data available on the BSE-risk linked to natural mating in cattle. In these circumstances mating will be restricted, in most instances, to heifers and cows on the same farm as the bull. At each service each female recipient will

receive the full ejaculate of semen into the anterior vagina. If any agent is present in spermatozoa or in the accompanying fluids the complete dose will be received into the anterior vagina. Live sperm will swim through the cervix into the uterus but usually only one will fertilise an ovum.

In regard to dairy heifers, dairy cows in bail herds and beef cattle bulls may be run with the females. Supervised natural service of dairy cows is more usual where artificial insemination (AI) is not used. In contrast to mating by AI, during natural service there is close contact between the bull and the female apart from the act of coitus.

Sometimes vasectomised bulls are used in herds to assist in the detection of oestrus. The same natural processes that occur during mating with entire bulls occurs, with the exception that no spermatozoa are transferred.

b) Artificial insemination (AI)

Bulls for artificial insemination usually undergo progeny testing before use. Stringent and regular health checks are carried out. Ejaculates are also examined microscopically before processing. In AI centres there is no contact between the bull and other females, other than with teasers.

Progeny testing for dairy cattle commences soon after puberty. A pre-determined number of females were inseminated with semen from the particular bull, following which the bull will usually be 'laid-off' and not used further until the results of the progeny test are known. The progeny are compared with those derived from other sires in regard to a number of production traits. Superior bulls are retained. Inferior bulls are slaughtered. The retained bulls re-enter the AI centre and semen is regularly collected for use. It is stored and transported in liquid nitrogen.

Semen is diluted in freezing medium in order to obtain a standard concentration of viable spermatozoa. The dilution factor will vary according to the cattle breed, the volume of the ejaculate and the concentration of the viable spermatozoa. The range of dilution, depending on these variables is from 1/3 to 1/100. The diluent used for conservation may either be free of proteins of animal origin or may contain lecithin obtained either from chicken eggs (Rousseau *et al* 1998) or bovine milk suitable for human consumption. If the diluent contains no added animal-derived product there is no added hazard or risk in regard to BSE. Any risk there may be from bovine milk or from chicken eggs is regarded as negligible.

Taking account of the fact that insemination straws contain 0.25 ml of diluted semen, a single ejaculate could be used to produce 30 to 1000 straws. Thus one AI bull could service this number of females from each ejaculate and many times this over a lifetime. All other things being equal, superior bulls are likely to service more cows than bulls of average quality and their semen is more likely to be exported.

Assuming there was a BSE risk in semen, this risk could come from spermatozoa or from the seminal fluids. Any risk from spermatozoa would depend upon the incidence of infection in the population and in the particular sperm that fertilised

the ovum. Any risk from the seminal fluid is considered likely to be evenly distributed throughout the volume of the ejaculate. Any risks there may be would be minimised for each inseminated cow by AI (in contrast to a cow receiving a full ejaculate from natural service) by a factor determined by the dilution, which in turn would depend on the sperm count and other factors.

The diluent used for sperm conservation may either be free of proteins from animal origin or contain lecithin obtained from eggs (Rousseau *et al*., 1998) or milk. If the sperm is diluted and frozen in a medium devoid of animal protein, its use will not add any further BSE risk.

However, no process used in bovine semen straw production is known to have any inactivating effect on TSE agents. Therefore, in this situation more recipient females would be a risk of exposure but the risk for each would be lower than from natural service. The contrast between the risks from semen delivered by natural service and by AI is analogous to that described by Gale, (1998) in regard to dilution effects. If the only significant risk was from the sperm that fertilised the egg, the incidence of infection in the population of spermatozoa would determine the risk from that insemination.

It is noted that a pregnancy does not result from every insemination, a live birth does not result from each pregnancy and that not all offspring are kept for the full incubation period of BSE. Furthermore cows are sometimes inseminated and mated by natural service at the same oestrus and may be inseminated with semen from different bulls at subsequent services or in different lactations.

III.I.2. Field Data

a) Sire-Calf Pairs of Confirmed BSE Cases in Great Britain

470 cases of BSE in bulls have been confirmed in Great Britain to the end of October 1998 (0.3% of all cases). **TABLE 4** below shows the recorded BSE status of the dam and sire of 39,175 BSE-affected animals born after the introduction of the ruminant feed ban in July 1988.

TABLE 4: RECORDED BSE STATUS OF THE DAM AND SIRE OF 39,175 BSE-AFFECTED CATTLE BORN AFTER THE INTRODUCTION OF THE FEED BAN (Status at the end of October 1998)

		BSE STATUS OF SIRE		
		+	-	unknown
BSE STATUS OF DAM	+	1	1,358	297
	-	19	22,844	6,343
	unknown	0	764	7,549

Further analysis of these data is warranted.

b) Published analysis of BSE risk from semen

The incidence of BSE in the progeny of the first two artificial insemination (AI) donor dairy bulls that were confirmed to have BSE has been compared with that in the progeny of two contemporaneously born dairy bulls whose semen was used in the same geographical area. The results of the comparison did not reveal an excess of risk of BSE for offspring of affected bulls (Bradley and Wilesmith, 1993).

c) Unpublished, incomplete analysis of risk of BSE transmission by semen – Preliminary results of a research project currently being undertaken by the Veterinary Laboratories Agency, UK.

The current data are on the offspring of 158 bulls used for AI. Nineteen bulls subsequently went on to contract BSE and 139 were healthy controls. The bulls in the two cohorts were born at the same time. The survival and BSE-status of all female offspring retained for breeding has been determined. There is no difference in the survival of the offspring between the two categories of bull.

The analysis of data from a somewhat larger study restricted to the progeny testing phase is still in progress. Semen was collected whilst the bulls were about 12 months of age. The analysis at present shows there is no enhanced risk of developing BSE for offspring whose sires developed BSE.

d) Semen exported from countries with BSE to other countries with BSE in native-born cattle.

No data have been published about semen, ova or embryos exported from countries with BSE in native-born cattle to other countries and particularly those with BSE in native-born cattle and any association there may be with disease occurrence. (See also the note in Paragraph III.C).

The Working Group strongly recommends that such data are made available and analysed.

III. I. 3. CONCLUSIONS

On the limited data available, it appears that the risk to offspring born to BSE-affected bulls is less than that of offspring born to BSE-affected dams. [7]Currently there is no enhanced risk of developing BSE for offspring whose sires developed BSE subsequently. It is noted that analyses have been mainly restricted to dairy bulls from whom semen was collected at a young age and early in the incubation period for the bulls that subsequently went on to develop BSE. However, in regard to offspring derived by artificial insemination (AI), it is noted that the majority of commercial inseminations result from semen obtained from dairy bulls over 6 years old, after progeny testing is complete.

[7] The Working Group had access to unpublished research results which were provided in confidence. The relative risk (Number of BSE cases in the offspring from BSE infected sires as compared to cases from non-BSE infected sires) did not indicate that semen was likely to be a significant route of infection.

Analyses similar to those performed for maternal risk assessment could be undertaken using the BSE case database in Great Britain.

III. J. RISKS LINKED TO EMBRYO TRANSFER

III. J. 1. Introduction

a) Veterinary medicinal products used for super-ovulation and oestrus synchronisation

These products have not been assessed as this is the province of those concerned with the safety of medicinal products.

b) Media for embryo flushing, washing, maintenance and transport.

Embryos are collected from donor cattle by a process of flushing. Flushing fluids are used for this purpose. For international transport of bovine ova, the protocols of the International Embryo Transfer Society and recommendations of the OIE are followed. Following collection ten washes are given. These same protocols may also be used for transfers within countries. Embryos can be directly transferred from the donor to one or more recipients, or they may be transferred after freezing at a different site and at a different time. Embryos, like semen are stored in liquid nitrogen.

Whatever the methodology used (direct transfer or transfer after freezing and thawing) the embryos are in contact with media containing proteins mostly of bovine origin. Only animal proteins of known and approved BSE-free sources should be used according to the rules defined by the OIE.

III.J.2. Experimental Data

a) Embryo Transfer Studies (CVL)

An embryo transfer study is currently taking place at the Central Veterinary Laboratory in Great Britain. Embryos were collected from BSE clinically-affected dams and transferred into BSE-free heifers imported from New Zealand. **TABLE 5** below shows the number of embryos transferred, the number of calves born and the BSE status of the animals at 1st July 1998.

TABLE 5. NUMBER OF EMBRYOS TRANSFERRED, RESULTING CALVES BORN AND ALIVE AND BSE STATUS BY DATE

Date of embryo transfer	Number of embryos transferred	Number of liveborn calves	Number of BSE cases at 1/7/98
Jul/Aug 1991	129	102	0
Jun/Aug 1992	175	114	0
Nov/Dec 1992	89	50	0

Ignoring natural survival, the likelihood for these data is given by:

$$[(1-p)+p(1-F(6.3))]^{102}\,[(1-p)+p(1-F(5.3))]^{114}\,[(1-p)+p(1-F(4.91))]^{50}$$

where p is the probability of infection and $F(x)$ is the cumulative distribution function of the incubation period of BSE. This gives a 95% confidence interval for the probability of transmission through embryo transfer of (0% - 1.5%).

b) Embryos exported from countries with BSE in native-born cattle to other countries.

No data are currently available on embryos exported from such countries to other countries.

III.J.3. CONCLUSIONS

Preliminary results from the incomplete embryo transfer study suggest a**n** extremely low risk of transmission (95% confidence interval 0 - 1.5%). These results suggest that direct maternal transmission is mediated later on in the gestational period or during or following birth of the animal rather than *via* the embryo.

IV. SUMMARY CONCLUSIONS OF THE WORKING GROUP

IV.A. ENHANCED RISK OF BSE IN THE OFFSPRING OF DAMS WITH BSE

There is an enhanced risk of approximately 10% of BSE in offspring born to BSE-affected dams. The results of all epidemiological studies undertaken to date have been consistent with a rate of direct maternal transmission of approximately 10%, in calves born to dams within 12 months of onset of clinical signs of BSE, with lower rates up to 24 months prior to the onset of clinical signs in the dam. Enhanced genetic susceptibility cannot be excluded on the basis of these data but such genetic susceptibility at present is only speculative. On the basis of these data the UK SEAC concluded that there is some evidence of direct maternal transmission at a low level but they cannot rule out variation in genetic susceptibility to feed-borne infection as an additional factor. It is thus still unclear if maternal transmission of BSE in cattle in the traditional sense occurs or not and if it does the mechanism involved. Even if it does occur the epidemic in the UK will not be maintained.

The WG strongly recommends that research would be started or strengthened in the fields identified in the previous sections as “deficits in knowledge”. Special attention should be given to:

a. the testing of the infectivity of semen, embryos, colostrum and milk of animals of various ages, without a species barrier and via the intra-cerebral route[8] of transfer.

b. epidemiological analyses of the available data on traded semen and embryos

IV.B. SEMEN

IV.B.1. Small ruminants

a) Scrapie

Sheep semen has not been proven to be a risk of transmitting TSE agents. The negative evidence is probably not as strong as that in cattle. The complexity of the epidemiology of scrapie, due to variation in *PrP* genotype and other possible sources of infection, make this a difficult subject to investigate and conduct experimentally. In any event all the evidence points to a generally low risk from semen compared with other risks, such as those from placenta, feed containing TSE-infected material (*e.g.* mammalian MBM) or the environment.

There are no published data on the infectivity of goat semen or male reproductive organs. Nevertheless any risks there may be are likely to be lower than for other risks, as specified for sheep.

b) BSE

Judgement cannot be made as there are no data.

IV.B.2. Cattle

a) Scrapie

Judgement cannot be made as there are no data.

b) BSE

All the evidence points to there being an extremely low risk from bovine semen. In regard to semen used for commercial artificial insemination transmission is unlikely provided no risk is introduced by using animal-derived material in the diluent and approved and validated HACCP principles are followed during collection, processing, storage and use. Whatever these risks are they are likely to be lower still if semen comes from clinically healthy bulls, not the offspring of BSE cases.

IV.C. EMBRYOS

IV.C.1. Small ruminants

a) Scrapie

[8] Regarding inoculating cattle i/c with milk, the following comment can be made: In practice it might prove to be more appropriate / useful to look (also) at certain fractions of milk and to use transgenic mice rather than cattle once the model is proven. The aim of the experiments should be to improve the confidence that milk and colostrum do not transmit BSE. Colostrum is more important in the context of protecting animal health and eventually eliminating BSE and thus a BSE source for humans.

Regarding sheep, results from experiments in the US and in Scotland give inconsistent, if not conflicting results and neither of the initial studies followed the recommended protocols of the IETS. One of the studies was conducted in an environment where natural scrapie existed in some of the sheep. Given the knowledge that placenta can be an infected tissue it is possible that embryos could be a source of infection though it is not the only way that infectivity could reach the placenta. It could get there *via* exposure of the dam by the oral route during gestation for example. It is concluded that embryos could be infected with scrapie but it is still not conclusively proven that they are or what the incidence might be. If genes have to be moved round the world, this is probably still the safest way to do it.

Regarding goats, judgement cannot be made as there are no data.

b) BSE in sheep and goats.

Judgement cannot be made as there are no data.

IV.C.2. Cattle

a) Scrapie

Judgement cannot be made as there are no data.

b) BSE

There is experimental evidence that transmission via embryos is unlikely provided IETS protocols are used. It is true that the large experimental study in the UK is incomplete though currently negative. Nevertheless, even if positive transmissions could be attributed to embryos in the final stages of the experiment, the risk would be reduced by ensuring that the donor and recipient animals were clinically healthy and not the offspring of BSE-affected cattle, and that IETS protocols and the recommendations in the OIE *International Animal Health Code* chapter on BSE were followed.

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