



SmithKline Beecham
Pharmaceuticals

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Independent Expert Report

TSE & VRBPAC Joint Meeting
July 27, 2000

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Independent Expert Report**

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**A DISCUSSION ON THE
TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHY (TSE)
RISK, FROM SOURCE MATERIALS DERIVED FROM
CATTLE, USED IN THE MANUFACTURE OF VACCINES,
FOR HUMAN USE**

with particular reference to considerations raised by the Agency

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1. Introduction

1.1 Objectives

The objectives are:

1. To discuss the transmissible spongiform encephalopathy (TSE) risk from source materials derived from cattle, used in the manufacture of vaccines, prepared for use in humans. (For a list of source materials used and assessed for TSE risk see **TABLES 1a and 1b**).
2. In particular to assess the TSE risk from source materials used:
 - in the preparation of master and working seeds,
 - master and working cell banks,
 - fermentation processes and
 - in the formulation of final products

(For a list of source materials used for these purposes see **TABLE 1b**)

3. As a result of considerations by the agency, to present the evidence supporting the absence of *in utero* maternal transmission of BSE and consequent negligible TSE risk in fetal calf serum derived from the offspring of clinically healthy cattle. (see the final Section of this document).

NOTE: *Throughout the document the generic risks resulting from BSE infectivity in bovine tissues are analysed rather the risk from these tissues only in certain geographic locations. This is because a geographical risk factor depends, not only on the actual occurrence and reporting of BSE in a country, but also on a number of historical risk factors and on the implementation and timing of risk management strategies that are incompletely known. Furthermore, even if in place, it is necessary to have formal knowledge that the risk management strategies are enforced, to the level required to minimise the risk, to an internationally agreed and acceptable level.*

1.2 Background

The manufacture of vaccines is a biological process. It usually involves the growth of pathogens, such as viruses and/or bacteria in media. This is followed by inactivation and purification of the pathogens. Animal-derived materials, particularly from cattle, are widely used in the manufacture of vaccines. Currently, some of these animal-derived materials cannot be reliably substituted by materials of other origins.

Vaccines are produced on the seed lot principle. For most licensed vaccines, the master seeds and master cell banks used for current manufacturing have been developed and characterized in the 1970s and 1980s and stored as such since that time

in order to sustain the continuous provision of consistent "working" materials for use in routine production runs.

Source materials required were chosen on the basis of the quality criteria applicable at the time. Thus bovine blood was often sourced from the UK, because of the high health status of UK cattle and the high quality of veterinary surveillance.

The appearance of BSE in UK cattle in 1986 (Wells *et al*, 1987), its subsequent occurrence in other countries of Western Europe with some isolated cases further afield, resulted in the progressive development (from 1988) of measures to control BSE. The announcement of a new variant form of Creutzfeldt-Jakob disease (vCJD) in 1996 (Will *et al*, 1996; Bruce *et al*, 1997), provoked a greater scrutiny of cattle-derived material in human and animal food, cosmetics, medicines and biological products, than hitherto.

BSE can be readily confirmed by *post mortem*, microscopic examination of the brain but it is not possible to detect infected, healthy animals during the incubation period, which on average is 60 months (range 20 months to possibly lifetime). This means that in the average situation, BSE may have been present undetected in a country for several years before it is discovered, by which time it is already too late to guarantee and maintain the safety of the products mentioned above. The uncertainty of detecting cases of BSE in countries with inadequate veterinary surveillance and resources, together with the difficulty of tracing exports of cattle and cattle products from countries with BSE to those without, makes it increasingly difficult to delineate safe geographical sources in regard to BSE. Therefore, the analysis of the TSE transmission risk by source of tissue is of considerable importance.

In human medicine it is a major objective to prevent disease. This can be achieved for some diseases by vaccination. In some circumstances, a consistently high vaccination coverage within the population at risk is essential. Before making a decision to change the source of materials used to make a vaccine, the following factors must be taken into account:

- That the quality of the new source material is superior
- That the efficacy, tolerability and safety of the vaccine made from the new materials is equivalent to, or improved from, that in the vaccine it replaces,
- The length of time taken to demonstrate this,
- The risk to human health resulting from any reduced vaccine coverage in the interim and,
- Any reduced uptake of vaccination due to potential public fear created by the announcement of the perceived, hypothetical or actual risks (if these are the reasons that stimulated the change of source materials).

The present document is based on in depth documentation collected in collaboration with SmithKline Beecham. It is comprehensive with regard to materials of animal origin known to be used in licensed vaccines imported into the USA. **TABLE 1b** lists cattle, sheep and human materials (including the mixed species used to produce tallow) which would fall into the categories identified by the FDA as being important for consideration in regard to a TSE risk in licensed vaccines imported into the USA.

1.3 Basis for the analysis

The discussion presented in this document has been preceded by an extensive, recent risk analysis conducted by the author.

The basis of this risk analysis was to identify the original source of material. If this is determined to present a negligible TSE risk (a zero risk for any material is not possible to prove), then so is any product derived therefrom, provided there is no contact with, or inclusion of any other animal-derived material that may be perceived to carry a TSE risk.

With respect to the safety of the final product in regard to TSE risks, this is determined by the safety of the raw materials, the effect of the processes of manufacture and the use to which the product is put.

It is important to recognise that items received by a vaccine manufacturer as raw materials prepared from animal material may have been processed in some way before receipt. This means there can be raw materials used by a supplier, that are used to produce their end product that is then purchased as 'source material' for the vaccine manufacturer. It is important for the risk analysis to trace the source back to its animal origin. In a perfect system there would have to be full knowledge of the species, the geographical source (by country, and herds of birth and production and country of slaughter), whether an animal source was healthy, dead or alive when the tissue was collected, if passed fit for slaughter for human consumption and fit for human consumption, the tissue, any animal additives to the tissue and the processes used in the preparation of the final product purchased as raw material by a vaccine manufacturer.

The precise provenance of some of the materials can be incomplete for some historical items. For example, some of the material used to prepare master seed or master cell banks was produced at a time, often many years ago, when the detailed information that is currently collected was neither required nor sought. Retrospective acquisition of the currently required data is no longer possible.

TABLE 1a: SOURCE MATERIALS: by species and tissue of origin

NAME	SPECIES OF ORIGIN	TISSUE
MRC-5 cell line	Human	Fetal lung
Vero cell line	Monkey	Kidney
Viral seeds	Pathogens isolated from non-neural, non-LRS	Human tissue
Bacterial seeds	Pathogens isolated from non-neural, non-LRS	Human tissue
Recombinant material	<i>Saccharomyces cerevisiae</i> <i>Escherichia coli</i>	
Bovine Meat extract	Bovine	Skeletal muscle
Ox blood	Bovine	Blood (live cattle)
Donor calf serum	Bovine	Blood (live cattle)
Fetal calf serum	Bovine	Blood (killed fetuses)
Haemoglobin/Haematin	Bovine/Porcine	Blood
Pancreatic extract	Bovine/Porcine	Pancreas
Skimmed milk	Bovine	Milk (live cattle)
Sheep blood	Ovine	Blood
Amino acids	Bovine/Porcine Human Chickens	Bones/skin Hair Feathers
Porcine stomach extract	Porcine	Pancreas/stomach
Casein/Casein peptone/ Casamino acids/Lactose Lactalbumin hydrolysate/ Galactose/Hycase/Casein Hydrolysate	Bovine	Milk
Sodium deoxycholate Choline chloride	Bovine	Bile
Gelatine	Bovine/Porcine	Bones/skin
Polygeline	Bovine	Bones
Primatone	Bovine	Spleen
Cholesterol	Sheep	Lanolin (wool)
Glycerol Tween (Buffer components include fatty acids)	Farm animal species	Tallow derivative

TABLE 1b: SUMMARY OF RAW MATERIALS DERIVED FROM CATTLE, SHEEP AND HUMANS (and including the special case of tallow) ASSESSED FOR A TSE RISK: by species of origin

BOVINE (domestic cattle) – blood, milk, muscle, pancreas, skin, spleen, bones, bile.

OVINE (domestic sheep) – wool, blood

MAMMALIAN FARM ANIMAL SPECIES - (mainly domestic cattle, sheep, goats and pigs other than those animals or tissues excluded by law) PROVIDING RAW MATERIAL FOR RENDERING, TALLOW PRODUCTION AND MANUFACTURING TO PRODUCE TALLOW DERIVATIVES.

HUMAN – hair, MRC-5 cell line, pathogens isolated from humans

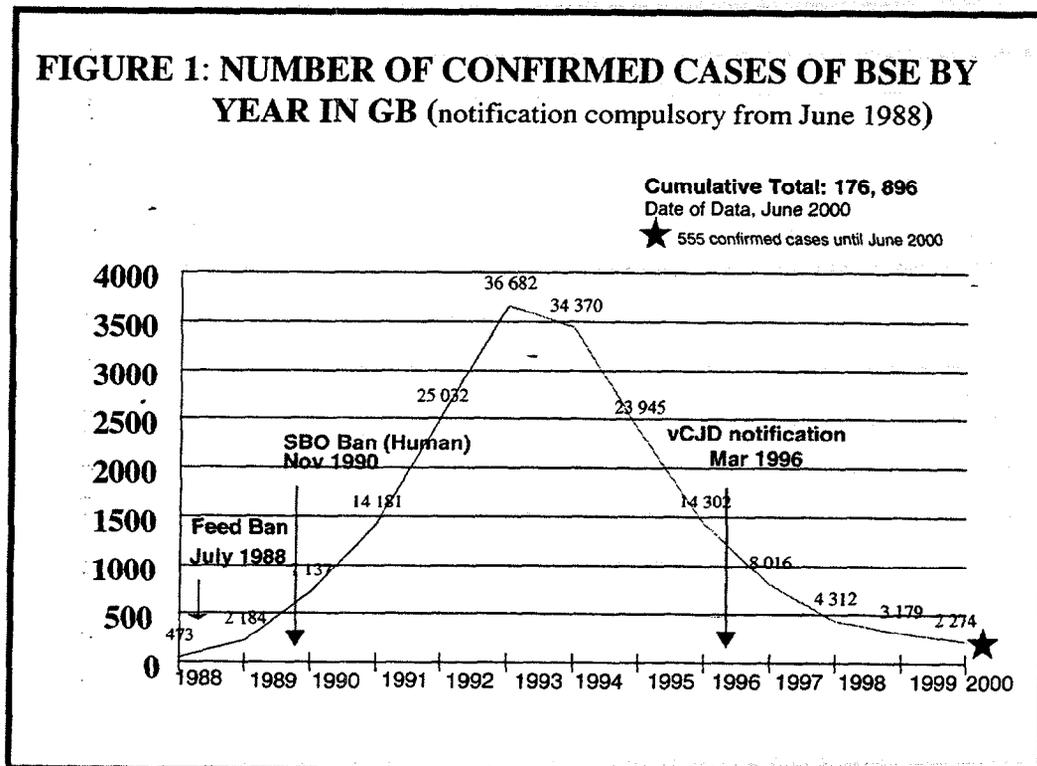
2. Epidemiology and pathogenesis of TSE, with emphasis on Bovine Spongiform Encephalopathy (BSE).

2.1 BSE in cattle

2.1.1 Epidemic, geography and timing

It is not possible to delineate the precise geographical distribution of cattle, cattle products (such as specified risk materials), and mammalian MBM, exported during the risk period from countries with BSE. Therefore, the analysis of the TSE transmission risk by source of tissue, collection method, process and use is of considerable importance.

BSE is a new, naturally occurring transmissible spongiform encephalopathy (TSE) of adult domestic cattle. It was first confirmed by microscopic examination of the brain of two dairy cows in England in November 1986. The first clinical case probably occurred in April 1985. Epidemiological studies on 200 cases reported by the end of 1987, suggested a scrapie-like agent was responsible and the vehicle for this agent was meat-and-bone-meal (MBM). MBM was incorporated into some concentrate cattle rations, notably of dairy calves. Vaccines used in cattle and a range of other factors were excluded as a cause. The mean incubation period for BSE is 60 months. Thus, the first exposures leading to the occurrence of clinical disease occurred probably around the period 1981-1982 (Wilesmith *et al*, 1988, 1991). **FIGURE 1** shows the number of confirmed cases of BSE in domestic cattle, by year, in Great Britain.



BSE in native-born cattle exists in several other countries of Western Europe including Belgium, Denmark, France, the Republic of Ireland, Liechtenstein, Luxembourg, the Netherlands, Portugal and Switzerland. The vehicle of infection is assumed to be the same as in the UK where it has been identified as meat-and-bone-meal (MBM) prepared by rendering animal waste (Wilesmith *et al*, 1991).

BSE has also occurred in other countries such as Canada (one case), the Falkland Islands (one case), Germany (six cases), Italy (two cases) and The Sultanate of Oman (two cases) as a result of importation of infected cattle incubating the disease from countries with BSE. However, so far as is known, all such cases have been identified, slaughtered and destroyed so they could enter no food or feed chain. Also, before a ban was imposed, mammalian MBM had been exported from the UK to other European countries, directly or indirectly and a small amount (12.3 tons between 1982 and 1985 and none thereafter, Walker *et al*, 1991) was exported to the USA.

Thus, in view of the impossibility to delineate the precise geographical distribution of cattle and mammalian MBM exported during the risk period from countries with BSE, the analysis of the risk of TSE transmission by type of tissue, source, collection method, process and use is of considerable importance.

2.1.2 Transmission and pathogenesis studies

The only tissues that show infectivity in cattle with natural or experimental BSE are CNS tissues and the distal ileum. Except for the distal ileum, no infectivity has been found in cattle up to 3 months before the onset of clinical signs. None of the tissues from which materials are derived for use in manufacturing of vaccines have shown any BSE infectivity. Neither the distal ileum nor any other part of the intestine is used in the manufacture of vaccines. The WHO and CPMP classification, based on observations of scrapie in sheep and goats, showing "low infectivity" (category III) for pancreas and "medium infectivity" (category II) for spleen are therefore not applicable to cattle potentially or actually infected with the BSE agent. In any case, even in sheep infected with scrapie there is no report of infectivity in pancreas during the incubation period but only in the clinical phase of disease (also in goats).

In transmission studies, tissues from naturally, clinically BSE-affected cattle were used to challenge BSE susceptible mice by parenteral (intracerebral [i/c] and intra-peritoneal [i/p]) inoculation. Infectivity was only detected in brain, cervical and terminal spinal cord, and retina. None of the ~ 50 other tissues tested (TABLE 2) have demonstrated any detectable infectivity (MAFF, 1999).

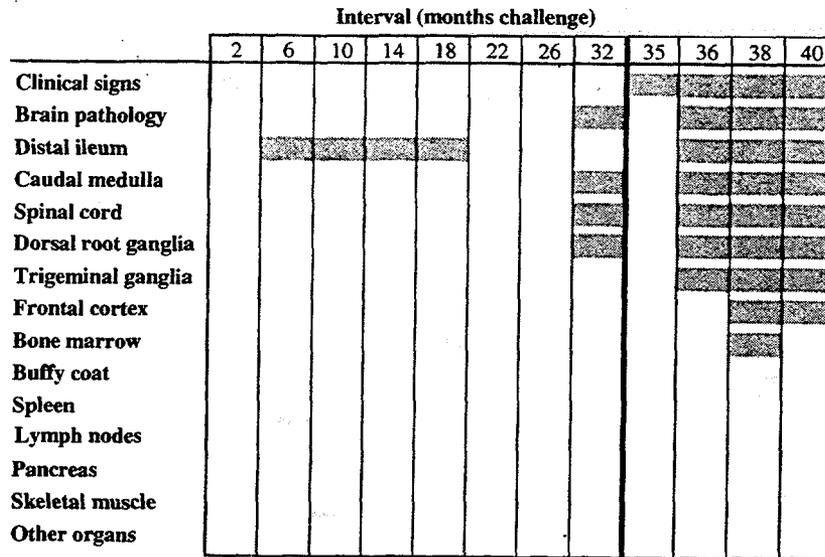
TABLE 2: BSE - TISSUES WITH NO DETECTABLE INFECTIVITY: MOUSE CHALLENGE BY PARENTERAL (i/c i/p) INOCULATION

SPLEEN	SKELETAL MUSCLE M. DIAPHRAGMA M. MASSETER	LIVER KIDNEY	TESTIS EPIDIDYMIS PROSTATE	OVARY UTERINE CARUNCLE
LYMPH NODES MESENTERIC PREFEMORAL RETROPHARYNGEAL	M. LONGISSIMUS M. SEMITENDINOSUS	PANCREAS	SEMINAL VESICLE SEMEN	PLACENTAL COTYLEDON AMNIOTIC FLUID ALLANTOIC FLUID
TONSIL	BONE MARROW	OESOPHAGUS RETICULUM		EMBRYOS
CAUDA EQUINA	BUFFY COAT SERUM BLOOD CLOT	RUMEN OESOPHAGEAL GROOVE PILLAR		FETAL CALF BLOOD
PERIPHERAL NERVES N. SCIATICUS N. TIBIALIS N. SPLANCHNICUS N. OPTIC	FETAL CALF BLOOD	OMASUM ABOMASUM SMALL INTESTINE PROXIMAL DISTAL		
CEREBROSPINAL FLUID	MAMMARY GLAND MILK MIDRUM FAT HEART SKIN	COLON PROXIMAL DISTAL RECTUM LUNG TRACHEA		

Source MAFF, 1999

To determine the temporal and spatial development of infectivity and pathology following oral exposure of calves to affected cattle brain, 30 calves were dosed orally at 4 months of age with 100 g of brain. Ten undosed controls were also incorporated into the study. Three challenged calves and 1 control were killed at approximately 4-month intervals commencing at 6 months of age. A range of tissues was collected at each stage for bioassay in mice. (Wells *et al*, 1999). **FIGURE 2** shows the infectivity of cattle tissues in mice over time. Except for distal ileum no other tissue showed detectable infectivity more than 3 months before the onset of clinical signs. The caudal medulla, the spinal cord and the dorsal root ganglia showed infectivity 3 months prior to the appearance of the clinical signs.

**FIGURE 2: CATTLE EXPERIMENTALLY, ORALLY-CHALLENGED WITH BSE:
Pathogenesis study in cattle, tissues bioassayed in mice**



No animals killed at 35 m post challenge

After Wells et al, 1999

Some tissues from selected stages of incubation or clinical disease are being bioassayed in cattle using the *i/c* route. Cattle are more sensitive than mice to BSE because of the elimination of the cow to mouse species barrier. Because no individual cattle experiment is concluded for seven years after inoculation (unless disease develops), the experiments are as yet incomplete. However, in this more sensitive model, where CNS tissues have already been shown to transmit disease, none of the tissues that showed no detectable infectivity in mice (including the buffy coat up to 3.5 years post-challenge), have so far transmitted disease to cattle (SAC Hawkins, personal communication).

The results of these experiments show that the WHO (WHO, 1997) and CPMP (CPMP 1992) classification, based on observations of scrapie in sheep and goats, showing "low infectivity" (category III) for pancreas and "medium infectivity" (category II) for spleen are not applicable to cattle potentially or actually infected with the BSE agent. In any case, even in sheep infected with scrapie there is no report of infectivity in pancreas during the incubation period but only in the clinical phase of disease (also in goats).

In conclusion, the only tissues that show infectivity in the case of BSE in cattle are CNS tissues and the distal ileum. Except for the distal ileum, no infectivity has been found in animals up to 3 months before the onset of clinical signs.

2.1.3 Evidence supporting the absence of TSE risk in fetal calf serum

There is no detectable BSE infectivity in fetal calf blood (bioassayed in mice) derived from a fetus of late gestational age from a clinically BSE-affected cow. This is regarded as a "worst case scenario" situation. By contrast, fetal calf blood is only collected in practice from fetuses taken from clinically healthy cattle, passed fit for slaughter and human consumption. Such fetuses are not exposed to either the birth canal or any post-natal, maternal source of BSE infection, if there is any. Fetal calf serum is derived from fetal calf blood and shares a similar negligible TSE risk

There is no detectable BSE infectivity in embryos or the placenta (bioassayed in mice and cattle). There is also no detectable BSE infectivity in the fetal fluids or maternal uterine caruncle from advanced pregnancy (bioassayed in mice).

Fetal calf blood from a fetus at an advanced gestational age from a naturally, clinically-affected cow with confirmed BSE was collected for bioassay in susceptible mice by the i/c and i/p route. Maternal uterine caruncle, fetal placental cotyledon, amniotic and allantoic fluids (and a large number of embryos collected from cattle with confirmed BSE) were also collected and inoculated similarly. No detectable infectivity was found in any of these tissues (Fraser and Foster, 1994, Bradley, 1999, MAFF, 1999). Embryos from the same source were also transferred into cattle and no transmission, either to the recipient cattle, or derived offspring has resulted after seven years, though the experiment will not be complete until 2001 (Bradley, 1996, Wrathall, 2000, A E Wrathall, personal communication. See also the final Section of this discussion for more detail of the embryo transfer experiment)

Two cattle with confirmed BSE and in advanced pregnancy, contributed placenta for bioassay in cattle by the oronasal route (Dawson *et al.*, 1991, 1994). No detectable infectivity was found in this study. Tissues from the inoculated cattle were subsequently bioassayed in susceptible mice and these also showed no detectable infectivity (SAC Hawkins, personal communication).

Fetal calf blood is necessarily collected *post mortem* and from healthy fetuses of healthy cattle, in advanced pregnancy, that are declared fit for slaughter and for human consumption. Such calves do not pass through the birth canal nor are they exposed to possible sources of BSE infection from their dam. Consequently two of the three possible routes of maternal transmission (see below and the definition in the final section of this document) are excluded as possible routes. This leaves only the *in utero* route as a possible route. However, this has not been positively determined and is not supported by any of the transmission studies reported to date. *See also the Section at the end of this document, before the Reference Section, which discusses evidence supporting the absence of in utero maternal transmission of BSE.*

2.2 TSE in other animal species and cross-species transmission

Naturally or experimentally induced TSE has been reported in various animal species. Sometimes a TSE agent derived from one species can induce a TSE in another species either naturally, or experimentally, provided an adequate dose is delivered by an appropriate route. The critical factors that determine if, and how readily, transmission occurs are the dose, the route of challenge or exposure and the strain of agent. It is not possible to predict the risk from a TSE agent from a particular species for another species.

2.2.1 Host range for naturally occurring TSE

Naturally occurring TSE have only been reported in a restricted number of species but since 1985 there has been a much larger range of animal incidents of TSE and the occurrence of vCJD in man. This recent extension to the natural animal host range for TSE has resulted from unnatural feeding practices in these species.

Naturally occurring TSE are described before 1985 in sheep and goats (scrapie - whose origin is from other sheep), deer and Rocky mountain elk (chronic wasting disease (CWD) - origin not known), farmed mink (transmissible mink encephalopathy (TME) - for origin see below) and after 1985, in cattle, domestic cats and a range of captive wild FELIDAE and BOVIDAE with SE (origin probably BSE from cattle) (Bradley, 1997).

2.2.2 Origin of transmissible mink encephalopathy (TME)

The origin of TME was for many years regarded as sheep with scrapie. However, in two outbreaks in North America (Canada and the USA) it has been suggested to be cattle infected with a rare TSE, rather than sheep with scrapie, because no sheep material was said to have been fed in these outbreaks (Marsh *et al*, 1991, Marsh and Hadlow, 1992).

To shed some light on the origin of this rare disease, experimental transmission of scrapie to mink by the oral route has been attempted and been unsuccessful (Marsh and Hadlow, 1992). Experimental transmission of scrapie to mink by the intracerebral (i/c) route is only sometimes successful and produces a disease indistinguishable from TME, though with a longer incubation period than seen in the natural disease. Thus there is some doubt that all outbreaks of TME result from exposure from sheep with scrapie. Experimental transmission of TME to cattle is successful and feeding the cattle brain back to mink transmits a disease indistinguishable from TME (Marsh and Hadlow, 1992). BSE also transmits to mink by the oral route but the clinical signs and the neuropathology are distinguishable from that in natural TME (Robinson *et al* 1994).

2.2.3 Cross species transmission of TSE

The species barrier

Any tissue that contains a given level of TSE infectivity and is inoculated by a defined route carries a greater risk of transmitting disease to another member of the same species than does an equivalent dose derived from a different species source, given by the same route. This is because with within species transmission, there is no species barrier. The strength of the barrier between animal species can be detected, and to some extent quantified, but the barrier to transmission of TSE between animals and humans cannot currently be determined because one cannot conduct the necessary experiments in the human species.

The species barrier is determined by two factors; the strain of agent and the variation in the *PrP* gene sequence between the donor and recipient species. It is unlikely that the species barrier between cattle and man for the BSE agent is zero (SSC, 2000). Nevertheless it cannot be quantified. Assuming that vCJD has arisen in some way by exposure of man to high titred tissues from cattle with BSE, this means that this particular species barrier can be broken.

Other factors

Two other factors contribute to the ability to transmit TSE from one species to another, namely the size of the infecting dose (a product of the weight or volume inoculated and the titre of infectivity per unit mass) and the route of exposure.

The intracerebral route is the most efficient route and the oral route is the least efficient. In some murine models the difference in efficiency between these two routes is up to 10^5 times (Kimberlin and Walker, 1988; Rohwer, 1996).

3. Tissues used in the manufacture of vaccines.

3.1 Introduction

This section is dedicated to the specific analyses of materials under consideration by the FDA and used in the manufacture of vaccines. Source materials of bovine origin (*i.e.* blood, milk, muscle, pancreas, spleen, bones, skin and tallow) and human origin (*i.e.* MRC-5 diploid cells, derived from human fetal lung and pathogens isolated from humans) are discussed in regard to risks of them carrying TSE infectivity. Materials of porcine, ovine, avian or any other species of origin that are used are not discussed. In particular, neither pigs nor chickens are known to have a naturally occurring TSE and neither species is susceptible to experimental BSE infection administered orally, the only likely route of exposure when if infected feed was fed. Thus, suffice it to say that there is negligible risk of TSE infectivity being transmitted in materials from these animals.

3.2 Materials of bovine origin

3.2.1 Blood and milk

- No TSE infectivity has been demonstrated in bovine milk, bovine blood, bovine blood derivatives or in foetal calf blood.
- Large volumes of bovine serum collected from the UK after 1985 have widely been used in the production of bovine vaccines. The distribution of these vaccines is not associated with the geographical distribution of BSE.
- There is a negligible risk of BSE infectivity being present in the fetal calf serum of fetuses derived from clinically healthy cows passed fit for slaughter and human consumption. Experimental and epidemiological studies do not contradict any of the other evidence supporting the absence of detectable *in utero* transmission of BSE.
- The stunning method and the use of pithing should be taken into account for those tissues that were collected from slaughtered animals, since contamination with neural tissues is a possibility. Due to the separate blood supply of fetuses however, the risk of contamination because of these procedures, is remote, in so far as it concerns fetal calf blood.

Serum (from donor calves or from fetal calves) is a substrate necessary for the growth of viruses. It is used in master and working seeds and in routine manufacturing. Milk derivatives are used as substrates for both viral and bacterial growth.

3.2.1.1 Blood and milk

TSE infectivity in blood has been reported in rodents following experimental intracerebral infection using agents other than BSE (Brown, 1995, Brown *et al* 1999).

It is unnecessary and unwise to translate these experimental findings to BSE in the bovine species when definitive results from bioassays of bovine blood are available as follows:

- In natural cases of BSE in cattle there is no detectable infectivity in buffy coat, serum, blood clot, spleen, lymph nodes, bone marrow or in fetal calf blood when bioassayed in mice by the *i/c* and *i/p* routes (MAFF, 1999). In addition a pool of spleens from natural cases of BSE showed no detectable infectivity after bioassay in cattle by the *i/c* route after seven years (pathology is awaited).
- In cattle experimentally, orally-challenged with BSE there is no detectable infectivity in spleen or lymph nodes.
- In cattle experimentally, orally-challenged with BSE there is no detectable infectivity in bone marrow during the incubation period of BSE. However, infectivity was found at one stage (of three) during the clinical period of disease

(see **FIGURE 2** and Wells *et al* 1999 for a full discussion on the possible reasons for this, which includes the possibility of a cross contamination event). It is noted that the WHO and the CPMP classify bone marrow (on the basis of studies done in natural sheep scrapie) as a CATEGORY III "low infectivity" tissue.

Furthermore, bovine serum is used in most viral vaccines administered to cattle (J Didelez, personal communication). In this case there is no species barrier. Due to differences in production processes between human and animal vaccines, the amount of serum in a bovine vaccine may be much higher than in any human vaccine (up to 150 µl/dose).

Several such vaccines are administered concomitantly. Between 1988 and 1990 around 10 million vaccine doses per year containing serum derived from UK cattle collected after 1985, have been distributed in Europe for use in cattle by at least one company (J Didelez, personal communication).

The greatest risk from BSE infectivity in blood, if it occurred, would be to cattle because there is no species barrier. Also the route of administration (s/c or i/m) is more efficient than the oral route, though clearly it is likely to be much less efficient than the i/c route. Out of the 29 million doses distributed between 1988 and 1990 (and produced with UK serum collected after 1985) only 2.4 million doses were distributed in UK (J Didelez, personal communication). The geographical distribution of reported BSE cases is thus clearly different from the distribution of those vaccines. Some of these animals will have been slaughtered for meat. However a significant proportion will have been maintained for longer as breeding animals. In addition epidemiological studies in the UK have shown that the BSE cases are not linked to vaccination (Wilesmith *et al*, 1988). These features point to the safety of bovine vaccines in the context of TSE transmission, whatever their ingredients.

No data have been found to suggest that bovine blood (from which milk is indirectly derived) could carry a different BSE risk than that for bovine milk. Bovine milk has been considered as safe by several independent organisations such as WHO, OIE, EC, SEAC (UK) and the US FDA despite the fact that it has a substantial cellular content, comprised, in part, of white blood cells.

3.2.1.2 Fetal calf blood and serum

Regarding the use of fetal calf serum the question on potential risk for maternal transmission has to be taken into account. Tissue transmission studies have not demonstrated any infectivity in any of the male or female reproductive tissues, or milk. In addition, an on-going study has not demonstrated the transmission of BSE by the bovine embryo (Bradley, 1996, Wrathall, 2000). Thus, there is no evidence for infection in any tissues that reasonably might be expected to be implicated in maternal transmission, if it occurred.

Furthermore, a range of epidemiological studies (covered in more detail in the final Section of this discussion), including a case control study (Hoinville, Wilesmith and Richards, 1995) and a cohort study (Wilesmith *et al*. 1997), do not provide positive evidence for the maternal transmission of BSE in the absence of a feed-borne source. Neither do they provide positive evidence for the *in utero* transmission of BSE or

provide any plausible explanation of how this could occur. None of these epidemiological studies set out to address the specific question of TSE risks in fetal calf blood or serum and in this regard are neutral. Collectively, these studies do not contradict any of the other evidence supporting the absence of detectable *in utero* transmission of BSE. (*This discussion is expanded upon in the final section of this paper*).

3.2.1.3 Stunning of cattle

Particular methods used to stun cattle at the point of slaughter can create an additional risk in blood from a clinically normal, infected cow where that infectivity had reached the central nervous system, by permitting brain tissue to enter the circulation. (**NOTE: As long as blood used from born calves or older cattle, is collected only from living animals, the risk referred to does not exist for this particular blood**). Results from a study of the pathogenesis of experimental BSE in cattle reveal that, in animals that develop clinical disease at 35 months of age, infectivity is detectable in the central nervous system some three months before clinical onset. It is unknown if particles of infected brain could traverse the capillary system of the lung (where most would be trapped) and enter the systemic circulation and thus possibly infect any organ. The particular stun guns creating the highest risk are those that inject air under pressure into the cranial cavity (Garland, Bauer and Bailey, 1996; Anil *et al*, 1999) that are not used to stun cattle for commercial purposes in the UK (Taylor, 1996; MLC, 1996). Captive bolt stunning, if followed by pithing, can have a similar but lesser effect (Anil *et al*, 1999). These methods of stunning and pithing should therefore be avoided wherever there is a risk of BSE. A European Commission Decision will prohibit pithing of all cattle for slaughter throughout the EU from 1 January 2001.

It is important to note that the previous paragraph does not apply to milk, bovine donor calf serum, ox blood or its derivative, serum, because all these materials are derived from living animals. Thus, under any circumstances milk, donor calf serum (derived from donor calf blood), ox blood and serum collected from a clinically normal, living animal have a negligible risk of carrying TSE infectivity.

In regard to fetal calf serum, the dam of the fetus may be stunned and pithed. However, the fetus would be unaffected by this procedure as it has a separate blood supply.

In the circumstances described, fetal calf serum presents a negligible risk of carrying TSE infectivity.

3.2.1.4 Conclusion

In conclusion, no TSE infectivity has been demonstrated in bovine milk, bovine blood, bovine blood derivatives or in fetal calf blood.

Large volumes of bovine serum collected from the UK after 1985 have widely been used in the production of bovine vaccines. The distribution of these vaccines is not associated with the geographical distribution of BSE.

There is a negligible risk of BSE infectivity being present in the fetal calf blood (and hence serum) of fetuses derived from clinically healthy cows passed fit for slaughter and human consumption. Experimental and epidemiological studies do not contradict any of the evidence supporting the absence of detectable *in utero* transmission of BSE.

When assessing TSE risks, the stunning method and the use of pithing should be taken into account for those tissues that were collected from slaughtered animals, since contamination with neural tissues is a possibility. Due to the separate blood supply of fetuses however, the risk of contamination because of these procedures, is remote, in so far as it concerns fetal calf blood.

3.2.2 Muscle, pancreas and spleen

Skeletal muscle, pancreas and spleen from clinical cases of naturally occurring or experimentally induced BSE in cattle have shown no detectable BSE infectivity following bioassay in mice for all three tissues and also in the more sensitive cattle model for spleen.

Bacterial growth media can contain several components of animal origin such as digested skeletal muscle extracts, pancreas or spleen extracts as well as blood, tallow, bone and milk derivatives. The present chapter deals with the BSE infectivity of muscle, pancreas and spleen.

Skeletal muscle from clinical cases of naturally occurring or experimentally induced TSE has not been shown to carry TSE infectivity following bioassay in susceptible animals in any species. Muscle is classified as a category IV tissue (no detectable infectivity) by WHO and the CPMP.

WHO (WHO, 1997) and the CPMP (CPMP, 1992) have classified pancreas in category III (low infectivity) and spleen in category II (medium infectivity) based on studies with natural scrapie in sheep and goats. Unlike in scrapie, no detectable infectivity has been found in either tissue, in either natural (MAFF, 1999) or experimental (SAC Hawkins, personal communication) BSE in cattle (including during any stage of incubation of experimental BSE) following bioassay in susceptible mice. Furthermore, BSE has not resulted in clinical BSE 86 months after challenge with spleen by the *i/c* route. All animals have now been killed and pathology is pending.

The comments on stunning and pithing of animals before slaughtering, described in the previous chapter are relevant here too.

3.2.3 Gelatin and amino acids derived from bovine bones and skin

The TSE risks in bones that have been historically used for gelatin manufacture may not have been negligible. However, if bones from cattle born, reared and slaughtered in the UK are excluded from the source used for gelatin manufacture, the residual risk in amino acids and 'Polygeline' derived from that gelatin, is regarded as negligible. Uncontaminated bovine skin from healthy animals, passed fit for human consumption can be regarded as presenting negligible risks for the production of gelatin.

During the course of the BSE epidemic the perception of risks from gelatin prepared from bones has changed. This is mainly because of the complexities of the different processes used for the preparation of gelatin from different source materials (skin and bone, cattle and pigs). Another reason is because of the potential inclusion of bones in the source material that came also from other species or from animals that had not come from animals passed fit for human consumption. Furthermore some bones could have been skulls or vertebrae that could have contained central nervous tissue. Such bones from cattle in geographical regions where BSE occurs could have been infected with the BSE agent, even if they had come from cattle slaughtered and passed fit for human consumption. During the course of the epidemic, improvements were made in regard to source, process and use, notably in the UK/EU.

Although the processes currently authorised for use in the EU are considered as safe in regard to TSE and for the purpose of producing gelatin, amino acids and other derived products for use in vaccine manufacture, these processes were not in place on a worldwide basis in the 1980s. At this time gelatin derivatives (see below) had been used during the generation of cell banks and viral seeds. These cell banks and viral seeds are currently used in most vaccine production on a worldwide basis. Gelatin manufactured in the UK, can no longer be used unless the bone is sourced from non-UK cattle.

The requirements of gelatin for its many uses demand a high quality product. This cannot be achieved if fat is present in the raw material. Thus degreasing bones with hot water under pressure has always been a commercial requirement as has acid demineralisation. The former has been shown to reduce the amount of specified nervous protein by 98-99% (Mantze *et al*, 1996). For cattle bones it is necessary to use a prolonged alkaline step in the subsequent processing as this has a more powerful inactivating action than the alternative acid process. Some authorities have specified the pH and time period that has to be used. (*e.g.* Commission Decision 96/362/EC). Even so, no-one has claimed that any process is completely effective at removing high titres of TSE infectivity. Nevertheless, the gelatin process is a harsh one and coupled with the significant dilution factors present it would seem to have been effective in preventing the transmission of TSE. The most sensitive indicators are cattle as there is no species barrier. Yet no case of BSE has been attributed to cattle despite large amounts of gelatin being fed to them *via* waste human food. One cannot totally exclude a risk from gelatin, but overall the historical risk is likely to be very small and currently is negligible.

Derivatives from bovine bone gelatin, including some amino acids and 'Polygeline', are used in the manufacture of vaccines. The authorised processes now used for gelatin manufacture give significant titre reductions when tested by spiking the raw material with high-titre scrapie agent before processing. It is concluded that amino acids prepared from bone gelatin would have a lower risk than the gelatin from which it is derived due to the additional processing required. Furthermore amino acids are not proteins and therefore by their very nature cannot exhibit the features of PrP and thus present a negligible TSE risk. (NOTE: *Historically, because the information was not collected, or is not now available, gelatin prepared from bovine bones (that may not have been specified precisely enough to exclude all bovine skulls and vertebral columns) may not all have been prepared by the most robust and controlled processes that are now used. Furthermore, the precise geographical origin of the bones from which gelatin was prepared (except for those used to produce 'Polygeline', see below) cannot now be determined with certainty, and thus an origin from cattle born in the UK, or other countries that now have BSE, cannot be excluded.*)

In regard to 'Polygeline', bones for the production of gelatin were collected in Germany, Austria and Switzerland in or before 1986. This is at least 4 years before the first confirmed clinical case in those countries (Austria, no confirmed cases, Germany only imported cases, first case in 1992; Switzerland, first case in 1990). Also, the processing conditions are reported to give a clearance factor in the range of $10^{9.2} - 10^{13.8}$ (Peano *et al.*, 2000). This figure is impressive but the authors do point out several features of their report that could be questioned. Firstly, they used high titre, hamster scrapie agent in the spikes, not the BSE agent or a murine-adapted strain. Secondly, they did not keep the hamsters beyond a year post-challenge and thirdly they added the successive titre reductions. Notwithstanding these criticisms the safety of 'Polygeline', under the circumstances described, is beyond dispute and thus presents a negligible risk of transmitting BSE infectivity.

Skin from cattle can be used for the preparation of gelatin, amino acids and other derivatives used during the manufacture of vaccines. Wool/lanolin, from domestic sheep, is a source of cholesterol also used in the production of viral vaccines. Bovine skin shows no detectable infectivity in bioassays (MAFF, 1999) and conclusions of the CPMP (1992) and the WHO (1997) list hair and skin in category IV (i.e. no detectable infectivity). One should however take care for contamination of the skin by infected material during stunning and slaughter.

In conclusion, the TSE risks in bones that have been historically used for gelatin manufacture may not have been negligible. However, if bones from cattle born, reared and slaughtered in the UK are excluded from the source used for gelatin manufacture, the residual risk in amino acids and 'Polygeline' derived from that gelatin, is regarded as negligible. Uncontaminated bovine skin from healthy animals, passed fit for human consumption can be regarded as presenting a negligible risk for the production of gelatin.

3.3 Special case: Tallow

Tallow derivatives using approved raw materials and procedures present a negligible risk of carrying TSE infectivity.

Mammalian farm animal species (mainly domestic cattle, sheep, goats and pigs other than those animals or tissues excluded by law) may be sent for rendering and the two products are MBM and tallow. Since tissues (including specified risk materials or SRM) from all species may have formed part of the material for rendering, care should be taken to specify what should be avoided. Rules to reduce risks have been developed (rendering parameters) in the EU. All countries in Europe that have reported BSE in native-born cattle have some form of SRM ban and the EC has recently introduced a uniform list of tissues from cattle, sheep and goats that will be classified as SRM in all Member States of the EU from 1 October 2000.

Tallow has not shown detectable infectivity whatever process is used (Taylor, Woodgate and Atkinson, 1995; Taylor *et al*, 1997). Tallow derivatives for use in biologicals, are prepared from tallow by processes that are accepted by disease control authorities as being sufficiently severe (*e.g.* 250°C, 50 bar, 3 hours followed by distillation at 200°C) to ensure safety in regard to TSE agents.

Tallow derivatives using approved raw materials and procedures present a negligible risk of carrying TSE infectivity.

3.4 Materials from human origin

3.4.1 MRC-5 diploid cell line, derived from fetal human lung.

The risk of the MRC-5 diploid cell line, derived from fetal human lung being infected with TSE agents is negligible.

The purpose of cell lines is to replicate viruses used in the manufacture of certain vaccines.

MRC-5 diploid cells were derived from a human foetal lung. The cell line was obtained from the National Institute of Biological Standards and Control (NIBSC) in the UK. The geographical origin of the human patient was the UK. The date of origin of the fetus that supplied the cells was 1965, some 31 years before the first case of vCJD was reported. The fate of the donor patient is not known but because she was of child bearing age the risk of being affected with sporadic CJD at the time or in the succeeding few years would have been very low. This is because the disease is rare and the sporadic disease is generally one that affects old patients. In addition, it is likely to be low for vCJD, because the date of origin was 1965, a time some 15 years before the first cows were exposed to BSE and 20 years before the first clinical case occurred in the UK.

No form of human TSE is known to be maternally transmitted, and to date there is no evidence that it could occur. Lung is classified by the WHO and CPMP as a Category III tissue, *i.e.* one with a low infectivity. However, this list is based on a range of data derived mainly from goats and Suffolk sheep with naturally occurring scrapie. Infectivity has been detected in lung tissue in natural and experimental CJD (Gibbs and Gajdusek, 1978, Masters, Gajdusek and Gibbs, 1980) but TSE agents are not known to replicate in human diploid foetal lung cells, and cell lines (including the MRC-5 cell line) have never been implicated in the accidental transmission of any TSE.

The MRC-5 cells were grown at the National Institute for Biological Standards and Control in UK (NIBSC) in a medium containing fetal calf serum. These cells have been distributed to various health authorities and European and US vaccine manufacturers.

In conclusion, taking account of all the available data, the risk of the MRC-5 diploid cell line, derived from fetal human lung being infected with TSE agents is negligible.

3.4.2 Pathogens isolated from humans

Pathogens used for preparing master seeds for each agent may be regarded as having a negligible TSE risk.

Pathogens isolated from humans are an essential starting point for the manufacture of many vaccines.

Various viral or bacterial agents causing human disease and used in vaccine manufacture have been originally sourced from infected humans. The epidemiology of the infectious diseases against which vaccines are prepared, indicates that the agents themselves are unable to transmit TSE at a detectable level, if at all. The risk of the pathogens, used in the manufacture of vaccines, carrying TSE infectivity is therefore remote and hypothetical.

In this context, pathogens used for preparing master seeds for each agent may be regarded as having a negligible TSE risk. The TSE risk from other materials used in the preparation of the master seeds is discussed below.

4. General comment on the dynamic nature of risk analysis

No risk analysis is static. As new data and information come forward appropriate new analyses need to be done. In addition to real risks there is also the concept of 'perceived risk'. This type of risk is exemplified by false risks voiced by some consumers and uninformed media comment. It is difficult to abolish this type of perceived risk. Avoiding the blighted raw material, if there are suitable and safe substitutes, is a method of avoiding the taint of a perceived risk.

Companies preparing biological products for human use are dependent upon the co-operation and like thinking of the various associated industries. For example, the gelatin industry provides raw material and there is no alternative source of this ingredient.

In order to eliminate perceived theoretical TSE risks, it may be necessary for example, to make changes in the cell banks and master seeds and to the process of manufacture. However, many such changes would require extensive validation and certification of the efficacy, tolerability and safety of the replacement vaccine.

5. Overall conclusions

1. The qualitative assessment of TSE risk in the raw materials used for the manufacture of vaccines has revealed no evidence for a degree of risk that is higher than negligible.
2. There is no means of replicating TSE agents that theoretically might be present in any raw material at a negligible level during the production process of any vaccine.
3. There is no evidence to suggest that the cumulative effect of all negligible risks could increase the level of risk in any product to higher than a negligible level.
4. Some vaccines require the use of cell banks or seeds which were developed decades ago. These do not contain any bovine material for which TSE infectivity has been demonstrated.
5. Despite the overall negligible risk there are possibly means of reducing that negligible risk still further by more strictly defining the specification of raw materials as indicated above.
6. The fundamental approach to avoid the risk of transmitting TSE agents from animals-to-man or from man-to-man, via biological products, is to secure safe sources of raw materials and whenever feasible, to use materials of non-animal origin that have themselves not been exposed to materials of animal origin during their preparation.
7. When deciding to modify the cell banks, master seeds or manufacturing process of vaccines, one has to take into account the necessity of guaranteeing the efficacy, tolerability and safety of the newly prepared vaccines.

6. A discussion on the evidence supporting the absence of *in utero* maternal transmission of BSE and consequent negligible TSE risk in fetal calf blood from which fetal calf serum is derived

6.1 Introduction

The agents causing natural scrapie of sheep are sometimes believed to be naturally transferred from one generation to the next and this is the reason why, once introduced, the disease often becomes endemic and difficult to eliminate. However, there is no evidence to show that in this species there is infectivity in the blood, or any component of blood of scrapie-affected animals at any stage of incubation. Neither has infectivity been reported in any of the tissues tested, from fetuses that were derived from Suffolk ewes with scrapie, (including brain, liver, thymus and spleen) (Hadlow, Kennedy and Race, 1982).

In an experimental study, Hadlow, Jackson and Race, (1984) inoculated 21 lambs *in utero* at 76-109 days of gestation with scrapie-infected brain material by the *i/m* route. Following birth, these lambs were sacrificed at 47 to 322 days of age and relevant tissues were inoculated into susceptible mice. Only three lambs showed any detectable infectivity, two at 254 days and one at 322 days of age. Infectivity was restricted to the suprpharyngeal, prescapular and mesenteric lymph nodes.

From the above studies, in a species in which infectivity can occur in the placenta, and where there is evidence of transmission from dam to offspring, it is concluded that transmission by an *in utero* mechanism is unanswered and not proved. In addition infectivity appears to be undetectable in the tissues of fetuses or lambs derived from clinically scrapie-affected sheep or in lambs experimentally inoculated with infectious agent *in utero*. Although the studies do not address the question of fetal blood infectivity directly, they collectively show that any infectivity that is present is undetectable and therefore at a very low titre, insufficient to generate infection in a susceptible species by the *i/c* route.

There is no other species (with the possible exception of goats) where natural or experimental transmission of disease occurs except by cannibalism. Even in sheep scrapie the precise method of transmission between generations is not known though it is known that the placenta of sheep can carry both infectivity (Pattison *et al* 1972, 1974, Onodera *et al*, 1993, Race, Jenny and Sutton, 1998) and PrP^{Sc}/PrP^{res} (Race, Jenny and Sutton, 1998).

6.2 Definitions of vertical, horizontal and maternal transmission

Vertical transmission is the transmission of disease from either parent either genetically or environmentally *via* germ plasm at fertilisation.

Horizontal transmission is transmission of disease from one animal to another by direct or indirect contact (including from dam to offspring during or after parturition).

Maternal transmission is transmission from dam to offspring *in utero*, during parturition or in the immediate post-parturient period.

From these definitions it can be deduced that unless a more precise definition is used there is an overlap between maternal transmission in the immediate post-parturient period and horizontal transmission. It is thus theoretically possible that so-called maternal transmission in some circumstances (including hypothetically in scrapie), could all be a form of horizontal transmission.

6.3 The question

This poses the question: is there a means of generation to generation transmission of BSE between cattle? and if so, does this pose a risk for blood derived from the bovine fetus?

6.4 The answers

There is a considerable body of information from transmission and epidemiological studies that supports the view that transmission of BSE from generation to generation in cattle either does not occur, or is rare.

6.4.1 The evidence from transmission studies

The evidence from transmission studies includes the following:

The following tissues derived from clinically-affected cattle with confirmed BSE have shown no detectable infectivity following bioassay in susceptible mice by the *i/c* and *i/p* routes:

- MALE and FEMALE REPRODUCTIVE TISSUES including:

Male reproductive tissues	Female reproductive tissues	Placenta
TESTIS	OVARY	PLACENTAL COTYLEDON
EPIDIDYMIS	UTERINE CARUNCLE	AMNIOTIC FLUID
PROSTATE		ALLANTOIC FLUID
SEMEN		
SEMINAL VESICLE		

Bradley (1996), MAFF (1999).

- EMBRYOS derived from clinically-affected cattle with confirmed BSE (inseminated with either semen from healthy bulls without BSE or semen from BSE-affected bulls),
- MAMMARY GLAND from clinically-affected cattle with confirmed BSE,
- MILK derived from BSE-affected cattle at three different stages of lactation has also shown no detectable infectivity. In this particular study, (Taylor *et al*, 1995) the milk was also fed to susceptible mice and no infectivity was detected. Furthermore, neither MILK + MAMMARY GLAND, nor PLACENTA, from a different group of cattle with confirmed BSE, fed to susceptible mice has resulted in transmission of a TSE (Middleton and Barlow, 1993).
- EMBRYOS derived from clinically-affected cattle with confirmed BSE have shown no detectable infectivity following bioassay in cattle. Neither has BSE occurred in any:
 - recipient cows after seven years or,
 - the resultant offspring also kept for seven years (Bradley, 1996, Wrathall, 2000).

This was shown in a comprehensive, within-species study (to be completed by 2001 when the last offspring is 7 years of age) involving 347 recipient cattle (337 already dead or killed, zero with BSE) and 266 embryo-derived cattle born alive (116 already dead or killed after birth, zero with BSE) using embryos for transfer collected during the clinical stage of the disease. This is claimed to be the time of greatest risk (Wrathall, 2000, AE Wrathall, personal communication).

- PLACENTA derived from clinically-affected cattle with confirmed BSE has shown no detectable infectivity following bioassay by the oronasal route in cattle after seven years, (Dawson *et al*, 1994, Bradley, 1996, SAC Hawkins, personal communication).

Conclusion

Collectively, the results of these studies indicate the absence of any means by which BSE could be plausibly transmitted from one generation to another.

In the context of the discussion the only form of maternal transmission that could conceivably involve infection in the blood is *in utero* transmission. However there is no transmission evidence to support this route of transmission.

6.4.2 The evidence from epidemiological studies

Bradley and Wilesmith, (1993) considered the possible role and significance of maternal and paternal transmission of BSE by the use of modelling studies. The model assumed that all offspring of BSE-affected cows would become infective irrespective of the time in the incubation period that calves were born. The results indicated that the effective contact ratio was less than 1:1, without which infection cannot be maintained in the cattle population.

In the epidemic in Great Britain a comparison is made between the observed annual incidence of BSE in offspring of confirmed cases and the expected incidence from the feed-borne source alone. In no year does the actual incidence exceed the expected incidence (Bradley and Wilesmith, 1993). It is now no longer possible to continue these observations since as a result of the export ban, the subsequent Florence agreement and other advice, various culls have been introduced that include culling all offspring of affected cattle confirmed to have BSE. There have been < 1900 out of a total of > 41,000 confirmed cases of BSE in the offspring of BSE cases born after the 1988 feed ban, yet over 7000 healthy offspring of BSE confirmed cases have been compulsorily removed from the food chain in 1998 and 1999 alone, as part of the Florence agreement. This indicates at best, a low occurrence of 'maternal transmission' of any kind.

MBM is definitively associated with the transmission of BSE in all countries where the disease has been reported in native born cattle. All such countries have had cases of BSE in their native-born cattle born after the ban in that country. Of a total of 1,283 cattle reported to have BSE outside the British Isles not a single one has reported a case in the offspring of a BSE case. This is despite the fact that we know that the strain of agent responsible in at least three countries is the same. In Switzerland for example, a country with BSE caused by the same strain of BSE agent as occurs in the UK, has had a total of 345 confirmed cases by March 2000. From this group of animals, brains from 182 offspring from 133 farms in 19 cantons were examined *post mortem* by microscopic examination according to methods described in the OIE Manual and for the presence of prion protein by immunohistochemistry. No evidence of BSE was found in any case (Fatzer *et al*, 1998).

In regard to paternal transmission this hypothetically, if it occurred, could enable the fetus to become infected *in utero* and thus potentially cause infectivity in the fetus. However, apart from the negative evidence of transmission from the bull by natural service and artificial insemination from infectivity studies on semen and embryos reported above, there is some additional epidemiological evidence supporting the view that this form of transmission is a negative factor in the transmission of BSE (Bradley and Wilesmith, 1993). Curnow and Hau (1996) using case control study techniques to compare the incidence of BSE in the progeny of two affected sires and 110 affected dams with the incidence in the progeny of non-affected parents all born before the feed ban was introduced. The results provided little, if any, evidence of differences in incidence of BSE in the different groups of progeny. For a full discussion on this topic see the Opinion of the EC Scientific Steering Committee (European Commission, 1999).

In regard to horizontal transmission on farms with at least one case of BSE over 30% have had only one case and over 70% have had less than five. Furthermore, the mean within-herd incidence has remained below 3% in any six-month period during the epidemic. These features do not support the occurrence of horizontal transmission.

A case-control study (Hoinville, Wilesmith and Richards, 1995) revealed that the offspring of cows that were subsequently affected by BSE were not more significantly found among cases (*i.e.*, dams that had confirmed BSE). However, there was a statistically significant risk for calves that were born up to three days after a subsequently affected cow calved (although it may not mean a causal association). In

any case this is not a plausible hypothesis for maternal transmission, particularly *in utero* maternal transmission, as there seems to be more risk for the calf of an affected cow contracting disease from another cow (after birth) than from its own dam. Rather, if it indicates anything it indicates negative evidence for maternal transmission. The authors conclude that feed or indirect (post parturient) sources are alternative sources of infection. Neither possibility could possibly make a case for fetal blood being infected. Nevertheless the results reported by the authors indicated they were consistent with a maternal transmission rate of 0% to 13 %.

The only evidence suggestive for maternal transmission comes from the cohort study (Wilesmith *et al*, 1997) but this study was unpredictably confounded by the continued risk of feed-borne exposure to infection, albeit at a decreasing level over time. Furthermore the study was not designed to find out the means of maternal transmission if it occurred or if fetal blood was infected if it did. Indeed, it has not been demonstrated that any form of maternal transmission of BSE in cattle occurs in the absence of a feed-borne source. Nevertheless, a commentary on this study is provided below.

In the cohort study, the animals were acquired from three annual birth cohorts (1987, 1988 and 1989). There was a declining risk of feed exposure from the first birth cohort to the last, that had the lowest risk. This is reflected in the similar declining incidence of the claimed 'maternal transmission' by cohort sequence. This strongly suggests a feed-related means of transmission that is concealed within the context of maternal transmission.

Further specific comments on this study have been made recently by Ridley and Baker, (1999). For example, the proposed hypotheses of maternal transmission and genetic susceptibility to environmental contamination both predict a greater number of affected calves amongst the offspring of BSE confirmed dams than amongst the offspring of BSE negative (at death) dams. The simple difference between 42 in the 'case' group and 13 in the 'control' group cannot distinguish between these possibilities.

Although the sample size (315 pairs in each group) was adequate to determine the simple difference between the two groups it was insufficiently large to take account of the confounding factors of feed exposure. An uneven distribution of contamination between participating farms also included a confounding factor.

All in all these authors conclude that the case for maternal transmission in BSE is not proved but rather consider it is a myth (Ridley and Baker, 1995).

Conclusion

In conclusion, although maternal transmission cannot be ruled out, it can be considered as most unlikely in view of all the available negative epidemiological and experimental evidence. Even if it did occur, there is no evidence for an origin in the absence of a feed-borne source. Furthermore, if it did occur, it cannot occur frequently, there is no evidence to support an *in utero* form of maternal transmission and no study provides the faintest glimmer of evidence that fetal calf blood and thus fetal calf serum carries more than a negligible risk of BSE infectivity.

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Private BSE Consultant

12 July 2000.

Annex 1: CV of Mr Raymond Bradley, CBE.

QUALIFICATIONS/EDUCATION/HONOURS:

- Veterinary Surgeon and Veterinary Pathologist
- MSc, BVetMed, FRCVS, FRCPath, CBiol, MIBiol

PROFESSIONAL POSITIONS (INCLUDING RELEVANT KEY POSTS/ACTIVITIES):

- Head of the Pathology Department at the Ministry of Agriculture, Fisheries and Food (MAFF, UK), Central Veterinary Laboratory (now called the Veterinary Laboratories Agency or VLA) from 1983-1991
 - Position held in 1986 during the discovery of Bovine Spongiform Encephalopathy (BSE) by two colleagues in the department.
 - Initiated and developed the initial BSE research programme with the aid of colleagues
 - Involved in the national and international issues concerning BSE and other transmissible spongiform encephalopathies of animals.
- BSE Co-ordinator for MAFF 1991-1995.
- Advisor to the WHO, OIE, EC, UK, Argentine and US Governments *via* Committees and Expert consultations on various aspects of transmissible spongiform encephalopathies (TSE)

ACTIVITIES SINCE RETIREMENT FROM VLA IN 1995:

- Private, independent BSE consultant.
- Advisor to the WHO, OIE, EC, UK, Argentine and US Governments *via* Committees and Expert consultations on various aspects of transmissible spongiform encephalopathies (TSE)
- Expert advisory positions for national and international trading associations representing various animal-based industries or companies.
- One pharmaceutical field consultancy, which is the present one with SmithKline Beecham Biologicals (SB Bio). My wide experience of BSE enables me to comment with authority on the TSE risk factors in animal-derived raw materials used in the manufacture of biologicals.

CURRENT MEMBERSHIPS:

- UK and Argentine TSE Committees.