



EPIDEMIOLOGICAL UPDATE ON BARB BSE CASES

1. By 31 July 1996 control measures had been reinforced resulting in the total ban on the sale or supply of any mammalian meat and bone meal (MMBM) or any feedstuff known to include MMBM. Following a recall scheme for feed containing MMBM, it also became an offence from 1 August 1996 to possess feed for farmed livestock containing MMBM. Cases of BSE detected in animals born after 31 July 1996 are known as born after the reinforced ban (BARB) cases.
2. The epidemiological features of the BSE BARB cases, from the surveillance of the first 59 BARB cases which were born and confirmed in Great Britain (GB) by 6th October 2003, will be presented to SEAC by Professor J Wilesmith.
3. Information on BARB cases was gathered from surveillance data on clinical suspects, casualty or emergency slaughtered animals, fallen stock and healthy slaughtered over thirty month scheme (OTMS) animals. For each case, one of the three EU approved screening tests was used and BSE was confirmed by immunohistochemistry, electron microscopy examination for scrapie-associated fibrils or modified Western blot, or a combination of these methods. A summary of the update is detailed below.
4. Wilesmith *et al.* observed that high incidence herds, in terms of the number of BSE cases per herd, were not represented disproportionately among the BARB BSE-affected herds. Wilesmith *et al.* propose that this, together with the occurrence of BARB cases in eleven of the fifty-seven affected herds that had not previously experienced BSE, suggests that environmental contamination is an unlikely risk factor to explain the occurrence of BARB cases.
5. Information on the dams of BARB cases with regard to their BSE status, survival and ultimate age indicates that a maternally-associated risk factor can not explain the majority of the BARB cases. There is no indication that BARB cases are localised in areas where BSE was prevalent one or more generation-lengths ago.
6. Wilesmith *et al.* note that one feature of the BARB cases is the marked reduction in the risk of infection with the BSE agent for cattle born after 31 July 1996 compared to the risk of infection in cattle born earlier. A second feature is the change in geographical risk of infection for cattle born after 31 July 1996, with risk becoming more uniform than in any other stage of the epidemic,

within this, however there is at present evidence of an elevated risk in south east (SE) England.

7. From the current analysis of the first 59 BARB cases, the markedly changed geographical distribution of BARBS compared to that of BSE cases prior to July 1996 is not consistent with a continued exposure from contaminated feed in previous high risk areas.
8. A feedborne source was proposed as an initial hypothesis for the first 16 BARB cases (Wilesmith, 2002). This hypothesis was based on the initial analysis that showed a continued difference in risk between animals reared in dairy herds compared to those reared in beef suckler herds, and lack of evidence for another source. An exogenous source was suggested because of the change in the risk of infection (geographically) for animals born after 31 July 1996 (such that this risk appeared to be more uniform across GB). Wilesmith *et al.* suggest that an exogenous feed source is plausible as significant amounts of ingredients for cattle feedstuffs have been imported and trading of MMBM was legally possible at ports within continental Europe until the European-wide feed ban of 1st January 2001. They suggest that the fact that cross-contamination of feedstuff ingredients has occurred in a number of other BSE-infected countries underlies this hypothesis. The reason for the apparently greater risk in SE England is not yet established but it is not inconsistent with an exogenous source from continental Europe, trans-shipped through European ports, imported through ports in SE England then used in cattle feedstuffs in this region.
9. Wilesmith *et al.* suggest that the difference in incidence between dairy animals and animals raised in beef suckler herds is not consistent with a genetically based origin, as all breeds appear to be susceptible to BSE. The authors also suggest that the incidence of BARB cases is too high to be compatible with a genetic cause such as mutation, as many countries with much larger cattle populations have not reported a BSE case. The occurrence of two pairs of cases in this small case series is also a strong argument against spontaneous mutation as statistically the chance occurrence of two pairs is unlikely. The occurrence and investigation of these two pairs of cases suggests a common exposure for each pair, for instance through a common feed batch.
10. Wilesmith *et al.* propose that the objectives of future epidemiological research should include investigation of exogenously-derived infection, endogenously derived infection and cross-species feeding, environmental contamination and horizontal transmission.

References

Wilesmith J.W. (2002) Preliminary epidemiological analyses of the first 16 cases of BSE born after July 31, 1996, in Great Britain. *Vet. Record* **151**, 451-452

Wilesmith J.W., Stevenson M.A., Morris R.S., Ryan J.B.M., Arnold M. and Prince M. (pre-publication communication) An epidemiological update of cases of BSE born after 31 July 1996 in Great Britain.



ORIGIN OF BSE IN RELATION TO BORN AFTER THE REINFORCED BAN (BARB) BSE CASES

Background

While the origin of the BSE remains unknown, the epidemiological evidence and effect of the feed ban(s) provides clear support that transmission of BSE in cattle occurred through infected meat and bone meal (MBM) in animal feed.

The inclusion of ruminant-derived protein in feed was banned in 1988 in GB (January 1989 for NI) and the ban reinforced on 31 July 1996. BSE cases declined dramatically in cattle born after the first food ban and it was hoped that reinforcement of the feed ban would eradicate the feed risk. However, it was anticipated that a small number of new BSE cases would be detected after the reinforcement of the ban due to maternal transmission of BSE. In Great Britain BSE has been detected in 59 animals born after 31 July 1996 (up to 6th October 2003). These cases are referred to as BARBs – born after the reinforced ban.

The origin of the source of infection and route of transmission for the BSE BARB cases remains to be resolved. Research shows that cattle are extremely sensitive to low levels of the BSE agent and it is possible that some residual cross contamination of food, from a presently unknown source, may continue to act as a source of infection. It is also possible that BARB cases emanate not from cross-contamination of feed but from other routes of infection which have only become evident as the feedborne source of exposure has reduced. Other sources of infectivity or “origin” of the BARB cases, for instance, maternal or horizontal transmission, genetic susceptibility or environmental sources of exposure have been proposed, remain plausible, but are as yet unproven.

When considering the origin of BARBs, it may be necessary to re-consider hypotheses about “factors that influence susceptibility to disease or infection”. While these hypotheses do not offer explanations as to the source of infectivity, it is conceivable that alteration of susceptibility to BSE could contribute (either alone or in combination with other factors) to maintaining the disease in cattle.

Many of the original hypotheses proposed for the origin and transmission of BSE may require further examination in the exploration for the source of infection for the BARB BSE cases. These hypotheses have been examined in detail in various reviews including the Phillips Inquiry on BSE, the Horn Review (*Review*

of the origin of BSE) and a European Commission Scientific Steering Committee (SSC) Opinion on “*Hypotheses on the origin and transmission of BSE*” adopted on 29-30 November 2001. Brief summaries of the hypotheses most relevant to this consideration are presented for information.

SEAC - review of BARB cases

When SEAC last considered this issue (September 2002), there were insufficient data to permit conclusions about the most likely source of infection for the BARB BSE cases. SEAC agreed that cross contamination of feed was a plausible hypothesis for the source of infection. However, other hypotheses such as the possibility of maternal transmission, albeit of a different form than had been postulated previously, genetic susceptibility and/or environmental transmission could not be eliminated on the basis of the current evidence.

Scientific Steering Committee Reviews on BARBs

The SSC reviewed this issue in November 2001 (*Opinion on the six BARB BSE cases in the UK since 1 August 1996*) and again in April 2003 when they examined the epidemiological features of the 34 UK born BARB BSE cases. No firm conclusions were made about the source of infection. The SSC agreed that the feed-borne hypothesis seemed to be supported by the evolution in the number of BARBs but that other possibilities could not be excluded (cross-contamination, maternal or vertical transmission, environmental contamination or spontaneous occurrence).

Feed-borne hypothesis as source of infection for BARBs

Removal of MBM from the food chain resulted in a dramatic reduction in the incidence of BSE in animals born after the feed ban. However, UK cases of BARBs continue to be detected since the reinforcement of the feed ban on 31 July 1996.

Based on the analysis from the initial 16 (now 59) UK BARB cases, Wilesmith *et al* (2002, 2003) propose that an exogenous (i.e. non-UK) feed-borne source remains the most likely source of infection. This hypothesis is based on the different risks of BSE detected in dairy herds compared to beef suckler herds. Wilesmith *et al* (2003) propose that an exogenous feed borne route of infection is plausible as trading of MBM was legally possible at ports in Continental Europe until the European-wide ban was introduced in January 1991. Significant amounts of ingredients for cattle feed have been imported into the UK over the last seven years and cross-contamination of feedstuffs and ingredients has occurred in a number of other BSE affected countries.

It is proposed that the change in geographical distribution of BARB cases, (greater risk in South East England compared with cases pre-1988 and 1988-96), is not consistent with continued exposure from contaminated feed in previous

high-risk areas in the UK. Wilesmith *et al* suggest that the increased risk in SE England is not inconsistent with a hypothesis involving an exogenous source, perhaps trans-shipped through European ports and imported through ports in SE England, being used in cattle feedstuffs. The case histories of the two sets of 2 BARB cases reared on the same farm suggest, it is possible, although not proven, that the animals could have received the same batch of feed.

Maternal/Vertical transmission

Early work suggests some support for maternal transmission (Wilesmith *et al.*, 1997). The incidence of BSE in offspring of cows, which developed clinical signs of BSE, was compared with that in offspring, born in the same calving season and herd, of cows, which had reached at least 6 years of age and had not developed BSE. All offspring were allowed to live to 7 years of age. The results indicated a statistically significant risk difference between the 2 cohorts of 9.7% and a relative risk of 3.2 for offspring of cows, which developed clinical BSE.

SEAC reviewed this study prior to publication and issued a statement 29 July 1996 <http://www.seac.gov.uk/statements/state29jul96.htm>. The committee concluded the study provided evidence that the risk of maternal transmission is in the region of 10% (confidence interval 5-15%) in the last 6 months of the BSE incubation period. However, under field conditions, only a fraction of BSE-infected cows giving birth would be within 6 months of showing clinical signs of BSE because of the long incubation period of the disease. The average transmission from cow to calf over the 60 months duration of infection in an animal prior to developing disease was estimated in the region of 1%.

An investigation of risk factors for BSE cases born after the 1988 feed ban (Hoinville *et al.*, 1995) reports that the findings could be compatible with rates of maternal transmission of 0-13%. The results from the maternal cohort study (Wilesmith *et al.*, 1997) are compatible with this finding and subsequent analyses of these data indicated maternal transmission at a rate of approximately 10% around the time of BSE onset in the dam (Curnow *et al.*, (1997) Donnelly *et al.*, (1997) and Gore *et al.*, (1997), with an analysis by Donnelly (1998) indicating a rate of 17.3%. However, it was considered that this rate of maternal transmission was unlikely to affect the future incidence and duration of the epidemic.

SSC reviews

In their opinion of 18-19 March 1999 "*Opinion on the possible vertical transmission of BSE*" the SSC concluded, regarding maternal transmission in cattle with BSE, that:

- *"the results of all epidemiological studies undertaken to date have been consistent with a rate of maternal risk enhancement of approximately 10% in the offspring of dams within 12 months of the onset of clinical signs of BSE. Where the time lapse between parturition and onset of clinical*

symptoms is longer than 12 months, the rate of maternal transmission is reduced. Whether infectivity is transferred directly before birth or after birth by a variety of mechanisms (e.g. calf infection by contaminated material, environment contaminated by blood, faeces, infected feed etc.) is uncertain and should be further investigated”

- *“There are no scientific data to support the hypothesis that infected calves are unduly sensitive to infection on a genetic basis”*

A later SSC opinion on *“Hypotheses on the origin and transmission of BSE”* in 2001 indicates that there is statistical support for some possible maternal transmission of BSE in cattle. If it exists it cannot account for more than 10% of the offspring of all cases with BSE, and probably less if the transmission to offspring occurs only if the dam is in the late stage of BSE incubation. The November 2001 SSC opinion concludes that there is no evidence that maternal transmission occurs in the absence of a feed-borne source and no plausible mechanism for maternal transmission has been identified in cattle.

The SSC opinion of 10-11 April 2003 *“BSE in United Kingdom’s cattle born after 31 July 1996 (BARBS)”* considered the 34 UK-born BARB BSE cases up to the end of Nov 2002. Although maternal transmission was ruled out in 9/26 cases that had sufficient information about the dam, in 8/26 cases, the dam died less than 12 months after giving birth to the BARB calf. However, in all of these 8 cases the dam’s death preceded active BSE surveillance so the dam’s BSE status is not proven. The SSC concluded that maternal transmission was not an explanation for those BARBS whose dam survived for at least 2 years after the birth of the BARB and survived to at least 6 years of age without clinical signs or a positive BSE test.

The most recent epidemiological analysis of BARB cases (Wilesmith 2003) reports that, in a study of the first 59 BARB cases, 72% of the dams of BARB cases, for which information was available, survived to at least 60 months of age. None of the dams had other offspring, which have so far developed BSE. It is proposed that a maternally associated risk factor cannot explain the majority of the BARB cases.

Embryo transfer research

In their opinion of 18-19 March 1999 *“Opinion on the possible vertical transmission of BSE”* the SSC conclude that *“preliminary results from the embryo transfer study suggest an extremely low risk of transmission (95% confidence limits: 0-1.5%)”* and that these results are *“consistent with maternal transmission being mediated later in the gestational period either during or following the birth of the animal”*. They also state that *“transmission of BSE via embryos is unlikely provided International Embryo Transfer Society protocols are used”*.

Since this SSC opinion was published, two further studies of embryo transfer from cattle affected by BSE have been published (Wrathall (2000); Wrathall *et al.*, 2002) indicating that the risks of transmitting TSE by reproductive technologies appear to be low, but caution is needed as research data are still limited. In the Wrathall (2002) study semen from bulls with clinical BSE were used to inseminate cows with clinical BSE and their resultant embryos collected seven days after AI. Viable embryos were transferred into recipient heifers imported from New Zealand. The recipients were monitored for 7 years after the transfer, and the offspring were monitored for 7 years after birth. None of the recipients or offspring showed signs of BSE. Histology, immunohistochemistry and electron microscopy for scrapie-associated fibrils were all negative. Infectivity studies with non-viable embryos in mice were negative. It was concluded that embryos were unlikely to carry BSE infectivity even if they were collected at the end-stage of the disease, when the risk of maternal transmission is believed to be highest. SEAC reviewed these studies in February 2002 and concluded that *“although the results had not shown that BSE could be transmitted by this route it was impossible to prove a negative. Although no risk had been demonstrated the results were not incompatible with a small risk of transmission”*.

Maternal fluids and tissues

Colostrum and milk

The hypothesis that BSE may be passed on in colostrum to calves after birth has been postulated by Clauss (2003). This stems from a report of a woman with CJD whose colostrum infected 2/10 mice at first, 5/10 mice at second and 21/21 mice at third passage.

In commercial dairy herds where the bulk of BSE cases arise, calves do not receive their dams' milk except for the first few days of life when they receive colostrum. In beef suckler herds it is common practice for calves to be suckled by their dams for up to six months. The SSC opinion of November 2001 states that colostrum from cattle with BSE has not been bioassayed. However existing data do not suggest that the rate of maternal transmission in beef suckler calves that have prolonged exposure to their dam's milk is any different to that in dairy herds where the only exposure to colostrum is in the first few days of life.

The SSC opinion of November 2001 *“Hypotheses on the origin and transmission of BSE”* reports that there is no evidence to suggest that milk is infectious for susceptible mice challenged by the oral or intracerebral route (Taylor *et al.*, 1995). Bradley (1999) did not detect any infectivity in milk.

In the statement of 29 July 1996, SEAC state *“There is no evidence from any of the transmissible spongiform encephalopathies that infectivity can be transmitted through milk”*.

Placenta

Only limited studies have been carried out. Bradley (1999) did not detect any infectivity in bovine placental cotyledon. Tuo *et al* (2001) found that although PrP^c was distributed widely in reproductive, placental and selected foetal tissues of sheep, PrP^{sc} was detected only in caruncular endometrium and cotyledonary chorioallantois of pregnant scrapie-infected ewes. In sheep PrP^{sc} accumulation in the placenta was found to depend on the foetal PrP genotype (Andréoletti *et al.*, 2002, Tuo *et al.*, 2002).

Semen

In the opinion of 18-19 March 1999 “*Opinion on the possible vertical transmission of BSE*” the SSC conclude: “*on the basis of the limited data available it appears that there is no enhanced risk of the development of BSE in the offspring of sires who developed BSE. It is therefore unlikely that semen constitutes a risk factor for BSE transmission*”. They also state that “*transmission of BSE by artificial insemination is unlikely for semen derived from BSE-affected bulls early in their incubation period*”.

Genetic mutation, genetic predisposition and sporadic BSE

Theoretically, it is possible that BARBs cases result not from “infection” but rather from sporadic genetic mutations (“sporadic BSE”) or genetic predisposition to infection. Wilesmith *et al* (2003) suggest that the incidence of BARBs is too high to be compatible with a genetic mutation. The difference in incidence between dairy animals and animals reared in beef suckler herds is not consistent with a genetically based origin as all breeds appear to be susceptible to BSE. Also many countries with higher cattle populations have not reported BSE cases and in addition, the observation of (two sets of) two BARB cases in the same herd presents a strong argument against spontaneous mutation, at least for these cases.

Evidence to date suggests that the incubation period or susceptibility to BSE does not differ between cattle breeds (Hunter *et al.*, 1994). Martin (*et al.*, 1991) genotyped the PrP gene in 103 animals, 16 of which subsequently developed BSE, but did not find an association between genotype and BSE. However the work of Neibergs (*et al.*, 1994) suggests that BSE affected cattle and their relatives are more likely to have a particular homozygous genotype than unrelated non-BSE animals of the same breed or animals of different breeds.

Ferguson (*et al.*, 1997) analysed the maternal cohort study (Wilesmith *et al.*, 1997) to examine if these results could be explained entirely by genetic predisposition to feed-borne infection rather than maternal transmission. The main conclusion of this analysis is that the results of the maternal cohort study are consistent with elements of both maternal transmission and genetic predisposition. This analysis suggests that the results of the cohort study could

be explained by genetic predisposition, acting in tandem with contaminated feed provided a large difference exists in the susceptibility of resistant and susceptible hosts.

Horizontal transmission

A study to examine possible horizontal transmission of BSE in cattle (Hoinville *et al.*, 1995) reported although there may have been an increased risk of BSE occurring in animals born on the same day, or between one and three days after an affected animal had calved, there was no plausible mechanism for this. However, when the SSC considered horizontal transmission as part of the opinion on “*Hypotheses on the origin and transmission of BSE*” in 2001, they concluded that for naturally occurring TSEs, horizontal transmission has been proven only in naturally occurring scrapie. A recent paper by Miller and Williams reports evidence for horizontal transmission of Chronic Wasting Disease (CWD) in mule deer (Miller and Williams 2003).

Risks from Environmental Contamination.

In examining the descriptive epidemiology of the BARBs cases, Wilesmith *et al* (2003) have proposed that high BSE incidence herds are not disproportionately represented among the BARB affected herds. This, together with the occurrence of BARB cases in 11 herds which had not previously experienced BSE, suggests environmental contamination is an unlikely risk factor to explain BARBS.

Various other hypotheses for “environmental contamination” have been proposed for the origin of BSE that could, hypothetically represent the origin of source of infection for the BARB BSE cases.

Theoretically, environmental contamination could result from the burial of animals that may have been infected with BSE. On-farm burial of fallen stock was not uncommon in the UK in the 1980s and early 1990s. Rapid testing of fallen stock was not then available and an unknown proportion may have been in the late stages of incubation of BSE. In this context it has been proposed that environmental contamination, for instance, from groundwater contamination, dispersal or transmission from animals/birds or accidental uncovering of buried carcasses could play a role in the persistence or transmission of BSE.

At present, it is not clear if sufficient exposure could result from environmental contamination as there is a paucity of data on the extent and rate of degradation of the BSE agent (or other TSE agents) in soil or groundwater following burial. Additionally the risk, if any, will depend on the infectivity in the carcass, circumstances, site and conditions of burial. One study published in 1991 reports an assumed infectivity reduction factor of 98% when supernatant fluid from scrapie-infected hamster brain homogenate was mixed with soil, packed into pots and buried for 3 years (Brown and Gadjusek 1991). Tateishi *et al* 1988 reported that CJD infected brain tissue remained infective after 22 months at room

temperature. A Defra research funded project is in progress to examine the potential leaching of prion protein through soil, however it will be several years the results of this research are available.

The SSC considered the risk of burial of BSE infected carcasses in their opinion published on 16-17 January 2003 on "*The use of burial for dealing with animals carcasses and other animal materials that might contain TSE/BSE*". The SSC concluded that the degradation process essential for BSE infectivity reduction is difficult to control and poorly characterised. The SSC confirmed that burial of animal material that might be possibly contaminated with TSE/BSEs poses a risk except under highly controlled conditions (e.g. licensed landfill). An earlier SSC opinion on the risk from burial of BSE infected carcasses from licensed landfills (2001) estimates that risks of contamination of water supply by leachate from licensed landfill to be below any level considered to be of significance.

During the foot and mouth epidemic in 2001, a working group of SEAC considered the potential risks of TSE infectivity in relation to the burial and incineration of foot and mouth disease carcasses. In this context, the working group considered that the number of BSE infected carcasses slaughtered for FMD control across all sites – let alone any particular site – would be small.

In 1997, SEAC reviewed an assessment of the risks associated with BSE via environmental pathways. This assessment suggested that the risks to human health were low, however the risks to animal health were not considered in this assessment. The Environment Agency is currently updating this assessment.

Risks from Sewage Sludge and Abattoir Waste

This concerns the theoretical possibility that infective prion agent may be passed from infected animals into sewage. As prions are highly resistant to degradation, it is proposed that prions survive the sewage treatment process. This could theoretically lead to re-infection of grazing cattle via the spreading of sewage sludge.

SEAC considered the practice of spreading of sewage sludge on land in the context of the risk of SRM particles from abattoir waste potentially entering the sewerage system. Provided the particulate matter was retained and disposed of as SRM, the committee was content for abattoirs to discharge their liquid waste to sewers and for sewage sludge to be disposed of by spreading on land. Any small particulate matter passing through the trap would be diluted to such an extent as to pose negligible risk.

Gale and Stanfield (2001) estimated the risks from sewage sludge based on the assumption that 1% of brain and spinal cord is lost to the sewer from abattoirs. The model predicts a risk of BSE transmission of $71 \times 10^{-5} \text{ cow}^{-1} \text{ year}^{-1}$ for cattle grazing on land to which sewage sludge has been applied. The authors conclude that the dose consumed by grazing cattle is insufficient to sustain the BSE

epidemic in the UK cattle herd. The risk from sewage sludge derived from human, cattle and other species remains theoretical.

From the SSC opinion of November 2001 "*Hypotheses on the origin and transmission of BSE*" it was suggested that waste animal by-products converted to organic fertilisers could historically have been a risk due to the possible inclusion of tissues from infected fallen stock, but once these are excluded the risks are regarded as low.

Concerns have been raised about the practice of spreading cattle blood and guts on land. In a statement of 7 June 1996, SEAC concluded that, there was no reason to advise against this practice as no BSE infectivity had been detected in blood and the evidence (at that time) did not suggest transmission occurred via horizontal transmission. It should be noted that it has not been permitted to spread blood from OTMS cattle since January 1997. SEAC were informed in June 2002, concerning new EU animal by-products regulations to come into force in 2003, that, for inclusion in fertilisers, mammalian blood would have to pass an ante-mortem inspection and be rendered to a pressure cooking standard. Under the new regulations, it would be prohibited to apply fertilisers containing blood or blood products on pastureland on which animals graze. The Committee were informed that the mammalian blood would originate from animals that were destined for the human food chain and as such would be classified as low risk. The Committee was "*content with the position although some concern was expressed over the potential recycling link of sheep blood, which could be distributed on fields where sheep may graze*".

Risk from tissues and excretions from infected animals

The likelihood that transmission of BSE can occur via exposure to excretions has been reviewed by the SSC in "*Opinion on 'Hypotheses on the origin and transmission of BSE'*". The SSC postulated from studies of scrapie in sheep and goats, that, if infectivity were present in excretions, it would be primarily from faeces, saliva, blood or urine. The SSC noted that in the case of naturally occurring disease, it would be less likely that feed or forage contaminated by faeces would deliver an infectious oral dose.

Blood

With regard to transmissibility from blood, Houston *et al.* (2000) showed it was possible to transmit BSE to a sheep by transfusion with whole blood taken from another sheep during the symptom-free phase of an experimental BSE infection. There is little information on cattle except for the study reported by Bradley (1999) that did not detect infectivity in cattle blood or blood components following bioassay by parenteral inoculation of mice.

Risks of transmission from other species carrying infection

Birds

The possibility that birds may act as possible transmitters of BSE was considered by the SSC (*opinion 7-8 November 2002 "Necrophagous birds as possible transmitters of TSE/BSE"*). The SSC concluded that birds could have theoretically ingested infectious material through fallen stock. It has been proposed that the spread of the ingested infectious material could occur through faecal contamination, as it is unlikely the pathological prion protein would be destroyed in the digestive tract. The SSC concluded that the possibility of active replication of PrP^{Sc} in birds is remote but agreed that such pathways of transmission cannot be excluded given these birds cover great distances during migration.

Rodents

Conception and Padlan (2003) have postulated that inadvertent ingestion of infected rodent parts, possibly droppings, may be a potential mode of transmission of TSEs. This postulate is based on sequence homology comparisons, which showed a close similarity between sequences of human and rodent prion proteins in a peptic fragment (that could result from gastric digestion) that corresponds a PrP fragment that is protease resistant and infective. This remains a postulate.

Other organisms

Transmission of TSEs through other organisms such as parasites and protozoa has been postulated. The limited research that has been carried out (in scrapie) suggests that mites may serve as a reservoir or vector for scrapie infectious agents. One study on hay mites in Iceland indicated that mites collected from 3/5 farms that had kept scrapie-infected sheep caused scrapie when parenterally inoculated into mice. PrP^{Sc} was demonstrated in the brains of these mice and also in mite concentrates from one of the farms (Rubenstein *et al.*, 1998). The theoretical risk exists that these organisms could harbour TSE infectivity and thus create a potential source or act as a vector of infectivity.

Alternative theory for BSE

Autoimmune theory

It has been proposed that BSE is an autoimmune disease (Ebringer *et al.*, 1997). The hypothesis suggests that a common organism found in soil, *Acinetobacter calcoaceticus* produces an immune response in cattle that, because of molecular mimicry, cross-reacts with myelin resulting in autoimmune damage to nervous tissue giving rise to BSE.

The autoimmune hypothesis was reviewed in the Phillips' BSE Inquiry which concluded that "the theory that BSE is caused by an autoimmune reaction is not viable". A similar conclusion was reached by the Horn review on the origin of BSE. The 2001 SSC Opinion on "*Hypotheses on the origin and transmission of BSE*" concluded there was not sufficient scientific evidence to support this hypothesis.

SEAC considered the autoimmune theory in February 2002 and concluded that, while the immune system may be involved in the initial replication of the BSE agent following peripheral exposure, it was not essential for the development of BSE infection. This is supported by studies showing TSE infection of immunodeficient mice after intracerebral inoculation of scrapie. Overall SEAC considered that the autoimmune theory was not a good postulate for the source of BSE.

Factors that increase susceptibility to disease or infection

A number of hypotheses have suggested factors that increase susceptibility to BSE such as exposure to organophosphates (OPs), deficiency in nutrients or various protective mechanisms or inadequate exposure to prostaglandins. These hypotheses remain speculative.

Organophosphates (OPs)

It has been proposed by Purdey (1992, 1994, 1996, 1998) that the use of OP compounds acted as the primary trigger that initiated the deformation of prion protein and the onset of BSE. This hypothesis proposes that the timing, distribution and dynamics of usage of OPs correlates with the epidemiology of BSE as well as accounting for cattle that developed the disease after the 1988 feed ban.

SEAC formally considered this hypothesis in 1997 but concluded that the hypothesis is incompatible with the epidemiological evidence of the BSE epidemic. Also, critically, the OP theory fails to account for the transmissibility of the disease.

It has also been suggested that the use of OPs increases susceptibility to infection with BSE. SEAC have reviewed the single experimental report, which suggests that the OP, Phosmet, increases the expression of cell surface PrPc in an *in-vitro* cell system. The committee concluded that it was not possible to draw conclusions on the basis of the available data.

The SSC considered the OP theory on a number of occasions (1998, 2001, 2003) and concluded that there was no scientific evidence of possible links between BSE and organophosphates used as pesticides in cattle. The SSC opinion on "*Hypotheses on the origin and transmission of BSE*" in 2001 states

that the OP hypothesis is incompatible with BSE epidemiology and does not explain the occurrence of BSE in Guernsey where no OP treatments were given.

Since the last SEAC review, a paper published by Wolferstan (2001) proposes that exposure of the dam to OPs prior to pregnancy, possibly exacerbated by mineral deficiency, might be the prime cause of the eventual development of BSE in her progeny. This hypothesis is based on a retrospective survey of OP, agrochemical, pharmaceutical use and BSE cases. The author proposes that the pattern of use of OPs as grain storage chemicals and pesticides from the late 1970s to the late 1990s was found to accord with the epidemiology of BSE. The author proposes that the reduction in use of OPs with the end of the warble fly, sheep scab eradication schemes and the introduction of the grain passport scheme could account for most of the decline in the number of BSE cases. However, an analysis of the agrochemical or OP dosage or frequency of exposure was not carried out, and there are flaws in the way the survey was carried out. These factors limit any possible interpretation of these data.

Mineral imbalances

Purdey (2000, 2003) has speculated that the mineral content of the diet may play a role in increasing susceptibility to disease or infection with TSEs. This is based on an analysis published by the author which reports a consistently higher concentration of manganese and deficiencies in a number of anti-oxidant co-factors such as copper in food chains “associated” with isolated clusters of TSEs (CWD in Northern Colorado, scrapie in Iceland). The SSC considered this hypothesis in their opinion of the origin of BSE and concluded

- “ *there are no localised clusters of CWD in Colorado or scrapie in Iceland that cannot be explained by the occurrence of conventional infectious TSE agents*”.

They added that:

- “*It cannot be totally excluded yet that manganese may be involved in competitive depletion of copper in certain important metabolic pathways including the brain and may thus increase the susceptibility of individuals to prion disease*”

In terms of the UK BSE epidemic, Purdey (2000) suggests that manganese (Mn) permeated the UK’s bovine food chain in the 1970s/1980s largely as a result of the widespread incorporation of chicken manure into the concentrated feed rations of cattle along with a range of Mn rich ingredients. Peak usage of fertilisers and fungicides high in Mn on fodder and forage crops occurred in the late 1980s/early 1990s. Purdey suggests that in environments where there is a high (Mn) concentration and deficiencies in antioxidant cofactors including copper there may be eventual depletion of copper binding to the prion protein PrP^c and excessive absorption of Mn by ruminants leading to substitution of Mn

at a vacant copper domain on the prion protein. This hypothesis was considered in the Horn report, which concluded that the hypothesis failed to account for the unique nature of the BSE epidemic and that, in England, the spatial distribution of copper and manganese concentrations in topsoil did not coincide with the BSE incidence.

A second stage of pathogenesis is also postulated by Purdey where intensive exposure to energy from, for example, ultraviolet sources (Purdey 2001) or acoustic shock waves (Purdey 2003) primes the prion protein into its pathogenic TSE isoform, hyperpolarised Mn^{3+} prions seed self-perpetuating clusters of neurodegeneration and TSE ensues. However, this remains unproven.

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