



SPONGIFORM ENCEPHALOPATHY ADVISORY COMMITTEE
Minutes of the open session of the 88th meeting held on 30th June 2005

At

The Conference Centre
Holiday Inn Bloomsbury
Coram Street
London
WC1N 1HT

Members: Professor C. Higgins (Chair)
Mr. J. Bassett
Dr. D. Brown
Dr. J. Chambers
Professor N. Hooper
Mr. P. Jinman (Deputy Chair)
Professor C. Lasmezas
Professor J. Manson
Professor I. McConnell
Ms. D. McCrea
Professor G. Medley
Dr. P. Rudge
Professor M. Stanley

Assessors: Mr. N. Gibbens (Defra)
Mr. A. Harvey (FSA)
Mrs. E. Lawrence (DH)

DA Assessors: Dr. A. Douglas (DARDNI)
Dr. M. Simmons (NAW)

Technical Advisors: Dr. P. Barrowman (Defra)
Dr. S. Dixon (FSA)
Dr. J. Stephenson (DH)
Dr. D. Matthews (VLA)
Professor J. Wilesmith (Defra)

SEAC Secretary: Miss K. Richards

Secretariat: Dr. T. Barlow
Ms. T. Dale
Dr. N. Ebenezer
Dr. P. Keep
Dr. V. Lund
Dr. C. Ravirajan

Also in attendance: Professor C. Bostock (Chair FSA/SEAC Milk Working Group),
Dr. J. Duncan (Member of FSA/SEAC Milk Working Group),
Dr. J. Hope (VLA) and
Dr. R. Jackman (VLA) for item 4.
Dr. Y. Boyd (Defra),
Dr. G. Saunders (VLA),
Dr. O. Windl (VLA) and
Professor W. Hill (University of Edinburgh) for item 6.

ITEM 1 – CHAIR’S INTRODUCTION

1. The Chair welcomed everyone to the 88th meeting of SEAC.
2. The SEAC Secretary explained to members of the public that it was the committee’s normal policy to conduct as much of its business as possible in open session. Holding open meetings allows the public an opportunity to observe the committee at work and provides an insight into how an advisory committee provides independent scientific advice to Government. A number of external experts and researchers involved in the studies that the committee will be considering are present. During the meeting the Chair will invite them to the committee table to present their work. Government officials are also present as members of the audience. As these officials are responsible for TSE policy in the various government departments they may also be invited to contribute to discussions.
3. The committee will also hold a closed session in the afternoon to allow discussion of unpublished research on atypical cases of scrapie. This is in accordance with the SEAC Code of Practice.
4. No apologies for absence had been received. Members were reminded that they are obliged to declare any commercial or other interests they may have in the agenda items. The next meeting would be held on Thursday 22nd September 2005 at the Church House Conference Centre, Dean’s Yard, Westminster, London.
5. The Chair informed members that Professor McConnell would be leaving the committee at the end of the August, upon completion of his second term of office. He thanked him for his enormous contribution to the work of the committee, including the Sheep Subgroup.
6. The Chair noted that interviews for new members had taken place. Recommendations from the interview panel would be submitted to Government Ministers for consideration.

ITEM 2 – APPROVAL OF MINUTES FROM SEAC 87 (SEAC 88/1) AND MATTERS ARISING

7. There were no comments on the minutes of the last meeting. They were agreed as a correct record.

8. The Chair noted that papers from the last meeting, discussed in closed session due to 'purdah' restrictions imposed by the General Election, had been published on the website after the election, as agreed.

ITEM 3 – CURRENT ISSUES

9. The committee was updated on the following issues:
- The Food Standards Agency (FSA) and the Committee on Microbiological Safety of Blood Tissues and Organs (MSBTO) had thanked SEAC for statements on a risk assessment on the age of vertebral column as specified risk material and the early phase of vCJD infection in blood recipients, respectively. The statements had been published on the SEAC website.
 - The 2004 SEAC Annual Report had been published on the SEAC website.
 - The Department of Health (DH) risk assessment of vCJD transmission via surgery, considered at SEAC 84, had been updated and published on the DH website.
 - A recent paper¹ had reported that the conformation-dependent immunoassay (CDI) for abnormal prions was more sensitive than other biochemical tests. Unlike most biochemical tests, it did not rely on proteinase K (PK) digestion of prions and could detect PK sensitive forms of abnormal prions. A member expressed caution about the assumption that the test was capable of measuring the infectious agent, as the form of prion constituting the infectious agent was still unclear. Dr Danny Matthews (VLA) explained that the CDI had been included in a ring trial of tests to evaluate the ability to discriminate prion strains. Unfortunately, evaluation of the test had been substantially delayed but was now underway, with results expected within the next few weeks. It was noted that a TSE Diagnostics workshop, organised by the UK TSE Joint Funders Group, would discuss advances in diagnostic tests in Autumn 2005.
 - An invitation for committee members to provide comment on the public consultation of the FSA Science Strategy 2005-2010 which is accessible on the FSA web site.

¹ Safar *et al.* (2005) Diagnosis of human prion disease. *Proc. Natl. Acad. Sci. U S A.* 102, 3501-3506.

- The TSE Joint Funders Group research strategy 2005-2008 will be published on the MRC website. SEAC had been consulted on the Strategy in 2003.
- Two clusters of three BSE cases born after the reinforced ban (BARB cases) had been recently identified. These will be discussed later in the meeting.
- Advice had been sought from the SEAC Chair by Defra about testing historic sheep samples. The Chair had responded that, in his opinion, analysis of these samples should not be rushed. A number of possible questions could be addressed, including genotype-phenotype interactions, whether BSE ever entered the sheep flock historically, and the possible historical prevalence of atypical scrapie. As Defra is still considering how to proceed, it was emphasised that research questions should be considered carefully before embarking on any studies, as the samples were valuable. Dr Matthews noted that EFSA had recently recommended that Member States test historical sheep and goat samples to help inform analysis of the phenotype and retrospective occurrence of TSEs.
- A member requested an update on the USA BSE case that had been reported recently, noting that initial results from tests carried out in late 2004 had been inconclusive, and follow up of the cases had taken a number of months. Given that removal of the Over Thirty Month Rule (OTMR) relies on a rapid determination of positive cases, the delay was of concern. Dr Matthews explained that BSE surveillance in USA had relied on preliminary testing using the BioRad ELISA, followed by confirmation of positive results by immunohistochemistry (IHC) only; other methods were not employed to investigate inconsistencies between the two tests. In the recently reported case, the animal had tested positive for BSE three times using ELISA but negative by IHC. The Office of the Inspector General in the USA had asked for further analysis to be conducted. Subsequently, Western blots had been employed in the USA, and by the VLA, on samples from the case. On the basis of these tests the diagnosis was confirmed as BSE. The VLA and American reference laboratory had also found the case positive by IHC on retest. An anomaly in the molecular weight of one of the bands in the Western blot had been noted, compared with that normally associated with BSE. USA BSE surveillance has since been altered to allow rapid confirmatory testing of

inconclusive results by a range of methods, including Western blot. It was noted that the birth location of the case was unclear. A member asked whether the CDI test was used for BSE surveillance in the USA. Dr Matthews responded that CDI had not yet been fully approved for use as a high throughput screen in Europe, and did not think it was in use in the USA yet.

ITEM 4 – RESEARCH ON ABNORMAL PRIONS IN BOVINE MILK (SEAC 88/2)

10. The Chair informed members that SEAC had, in 1997, concluded that there was no evidence that BSE infectivity could be transmitted through milk. However, due to continuing uncertainty and concern about potential low levels of BSE infectivity in milk, the FSA had commissioned research in 2002 to develop diagnostic tests to detect BSE infection-associated abnormal prion protein (PrP^{BSE}) in cows' milk and to screen milk which had been collected previously from cattle experimentally infected with BSE for the presence of PrP^{BSE}. The research had been overseen by a joint FSA/SEAC Milk Working Group (Milk WG), the terms of reference of which are set out in paper 88/2.
11. Mr Alan Harvey (FSA) noted that the study had been difficult to conduct and thanked the Milk WG for its perseverance in taking the work forward. He noted that the study had been unable to detect the presence of the PrP^{BSE} in the milk of infected cattle. In light of these results, and as a result of new research priorities, including a mandate to develop a diagnostic test for BSE in live animals, the FSA had decided not to take this work on milk any further. The committee was asked to comment on this decision.
12. Professor Chris Bostock, Chair of the Milk WG, set out the key results and the approaches taken to addressing the challenges posed by the research. The main challenge was how to validate the BioRad Platelia ELISA test for detecting PrP^{BSE} in milk, when only a limited number of presumed negative samples were available. The approach taken was to develop and apply two independent tests (based on completely different properties of PrP^{BSE}), of similar sensitivity. The BioRad Platelia ELISA, which normally employs PK digestion, was used as a screening test and 'reactive' samples were then subjected to confirmatory analysis using the Seprion-PAGE/Western blot test. This test employs specific binding of PrP^{BSE} to a ligand rather than the property of relative resistance of PrP^{BSE} to PK digestion. A sample would

have to test positive on both tests before it was considered positive for PrP^{BSE}.

13. Milk samples were collected from lactating cows which had previously been orally challenged with 1g (low dose group) or 100g (high dose group) of BSE-infected brain homogenate, and from an unchallenged, unexposed control group. All the animals calved at around 2 years of age, 18 months post challenge, and annually thereafter up to four lactations. Colostrum and milk samples were collected in the first week post calving, and milk samples collected regularly thereafter.
14. For determination of the limit of detection and dynamic range of the assays, negative milk samples were spiked with brain homogenate from BSE-infected cattle, pre-diluted in brain homogenate from cattle uninfected with BSE. To address the possibility that PrP^{BSE} in milk would not necessarily respond to PK digestion in the same way as PrP^{BSE} in solid tissue, and that milk could possibly contain PK-sensitive forms of PrP^{BSE} that may remain undetected, the BioRad Platelia ELISA was additionally employed without PK digestion of the samples. A higher incidence of 'reactive' samples in colostrum (as opposed to milk) was noted and in all samples when the PK step was omitted from the assay protocol. Twenty eight reactive samples were identified, 12 of which (all colostrum) initially gave positive results in the Seprion-Western blot test, but which were subsequently shown to be falsely positive through non-specific staining. The researchers concluded that none of the samples contained a detectable level of PrP^{BSE}.
15. The committee noted that the form of the abnormal protein in milk, if present, is unknown and that the cellular fractions (obtained by centrifuging milk samples) were chosen for testing because previous tissue fractionation studies on prions had shown them to be associated with cell membrane fractions. The committee noted Dr Jim Hope's (VLA) explanation that these previous studies provided a strong rationale for the current approach which assumed that PrP^{BSE} would sediment with cell membrane fractions.
16. The committee questioned whether the centrifugation process (500g for 2 hours) would sediment all of the cell membrane material and therefore whether there could be some membrane material (and prion protein) in the non-cellular fractions. The committee noted the reasons for choosing this centrifugation protocol and noted that the Milk WG had advised against

analysing the non-cellular (supernatant) fractions since this would have required complete redevelopment and revalidation of methods for these types of samples. In addition, it was unlikely that PrP^{BSE} would be present in the supernatant fractions while being undetectable in the sedimentable fractions.

17. The committee noted that there was no association between 'reactivity' of samples (tested by BioRad ELISA) and BSE challenge status of an animal. There was no correlation with increasing lactation number (which would have related to progression through the BSE incubation period) and no correlation with occurrence of mastitis (indicating that mammary gland inflammation does not appear to be linked to increased amounts of PrP in the cellular fraction of milk). There was, however, a higher incidence of 'reactivity' in colostrum samples compared with milk samples.
18. The committee noted that 28/541 samples, irrespective of PK treatment, were BioRad Platelia ELISA 'reactive'. These 28 samples were then subjected to the confirmatory Seprion/Western blot test. Twelve stained positive, however this occurred whether or not the primary PrP-specific antibody was present. In response to a question on whether staining in the absence of the PrP-specific antibody could be due to cross-reactivity between PrP^{BSE} and the secondary antibody, Dr Hope explained that a number of experiments had been done to exclude this possibility. He also noted that the Western blots had been repeated many times and that the consensus interpretation was that the staining was indeed not PrP-specific. It did not, therefore, indicate the presence of prion protein.
19. The committee noted that individual, rather than pooled, milk samples had been analysed. Therefore, if mastitis in some animals had led to abnormal protein being present in large quantities in milk this had not been missed due to a dilution effect.
20. The committee noted that there is no published evidence for the presence of BSE infectivity in udder tissue.
21. A member commented that, based on the sensitivities of the BioRad Platelia ELISA and Seprion-Western blot tests, the maximum infectivity that could be estimated to be present in milk was 0.05-0.14 Cattle Oral LD₅₀/litre. The committee noted that it might be appropriate to translate this estimate into an estimate of the maximum risks of transmitting BSE via milk into cows or into people.

22. SEAC considered the study to be well designed and carefully conducted. The committee agreed that the study showed no evidence for the presence of PrP^{BSE} in milk of experimentally infected cattle, within the limits of detection of the test methods used.
23. In conclusion, the committee:
- suggested that, for completeness, the soluble (supernatant) fractions might also be tested for PrP^{BSE} although it was recognised that, if it were decided to undertake this work, this would require some method development and validation
 - suggested that the study samples be retained to allow possible future analysis
 - concluded that the results of this study, together with the findings of previous epidemiological and experimental research (particularly no specific persistence of BSE in suckler herds), provided no evidence for the presence of PrP^{BSE} in, or for transmission of BSE via, milk.

ITEM 5 – DIFFERENTIAL DIAGNOSIS OF BSE (SEAC 88/3)

24. By way of introduction, the Chair mentioned that at previous SEAC meetings members had noted that a decreasing proportion of cattle identified by surveillance as showing clinical signs attributed to BSE had been confirmed subsequently as BSE positive. SEAC had asked Defra what investigations are being pursued to reach a diagnosis of disease in such cattle.
25. The Chair invited Dr Peter Barrowman (Defra) to discuss the approach taken by Defra to differential diagnosis and to enable the detection of atypical forms of BSE or other unknown or associated diseases in cattle. Dr Barrowman introduced Paper 83/3, which outlined the steps that Defra could take in regard to differential diagnosis and phenotype stability of BSE in cattle in the UK.
26. Dr Barrowman informed members that it was not possible to reach a conclusive clinical diagnosis in all BSE suspects. The diagnosis erred on the side of caution, such that uncertain cases would be classed as probable BSE to ensure BSE would not be overlooked. This continues to be a challenge as residual BSE cases decline, but other neurological cases continue to occur in the cattle population. Defra would take measures to ensure clinical expertise regarding BSE is maintained in the field.

27. Members were reminded that diagnosis of BSE in cattle is confirmed on post mortem (PM) samples taken from the obex of the brain. If the PM tests are shown to be negative for BSE no attempt is made to reach a definitive diagnosis. However, in individual research projects during the BSE epidemic, 40% of suspect cases ultimately do not show neuropathological changes. For those that do exhibit neuropathological changes, few can be linked to known aetiologies. For the reasons outlined in 83/3, Defra does not consider it feasible or justifiable to reach a differential diagnosis across the whole spectrum of suspect cases where BSE has been excluded.
28. Dr Barrowman stated that, in the UK, studies to date show a uniform clinical and neurological pattern to the cases of BSE examined and that it appears to be a single disease. He informed the committee that at the present time the majority of cases were detected by active surveillance, and with a rapidly reducing number of suspect cases, the opportunity for prospective studies are limited. Identification of a new variant or strain of BSE depends on defining the disease by case history, clinical characteristics, neurology, pattern of PrP^d deposition, and PrP^d signature. Current physicochemical and immunological methods can distinguish known BSE from other TSEs, however molecular characterisation methods are by themselves not developed sufficiently to classify new phenotypes of BSE in cattle. Should any changes in BSE phenotype occur, the need for modification of control measures to eradicate the disease and protect public health would be assessed.
29. A member pointed out that 'atypical' scrapie shows different patterns of prion deposition in different regions of the brain than classical scrapie, and in some cases does not result in a clinical phenotype. This should be borne in mind in considering how to detect atypical BSE should it arise.
30. A member suggested that a degree of caution was necessary when relating changes seen in brain tissue in laboratory tests to the clinical phenotype. A member suggested that, as carried out in one research study, it might be possible to carry out PMs in, for example, 100 BSE suspects as sentinels to detect any phenotypic changes occurring. In response to a member's query, Dr Barrowman and Dr Matthews informed the committee that in 2004 there were 351 suspect BSE cases reported, of these 310 were slaughtered and only 82 were confirmed cases of BSE.

31. The Chair considered that there were two important issues. The first was whether the undiagnosed cases indicated disease other than a TSE: this was not in SEAC's remit to consider. The second issue was whether a proportion of cases considered BSE-negative might in fact be atypical BSE. An important question was whether Defra could identify, by surveillance and improved diagnostics, any new variant of BSE or atypical case. Dr Barrowman stated that Defra is using recently developed diagnostic tools for this purpose where suitable material is available from current cases, although this is restricted to clinical cases as limited tissue is available from cases ascertained through active surveillance.
32. Dr Barrowman informed members that a training video had been commissioned to ensure the continued satisfactory clinical diagnosis of BSE in the field and should result in maintaining the expertise necessary for the identification of clinical cases. It would also allow the differential diagnosis in these suspect cases to be assessed to a greater degree.
33. In conclusion, the committee:
- acknowledged that, given the large number of clinical conditions that might resemble BSE, it was disproportionate to attempt to definitively diagnose all suspect BSE cases that are not confirmed as BSE
 - acknowledged the measures taken by Defra to ensure that BSE ascertainment is maintained in the field
 - emphasised that it is not unreasonable to expect that 'atypical' cases of BSE might arise, given reports from other countries and recent evidence for 'atypical' scrapie
 - considered it was crucial to ensure that atypical cases of BSE, should they occur, would not be missed. With this in mind, it would be appropriate to collect appropriate tissue samples in addition to obex, and to ensure application of the most appropriate methodology as it develops.

ITEM 6 – BORN AFTER THE REINFORCED FEED BAN (BARB) CASES (SEAC88/4)

34. SEAC considered three topics related to BSE cases born after the UK reinforced mammalian meat and bone meal ban in August 1996. Around 100 of these BSE cases, referred to as BARB cases, have been reported in the UK.

Sequencing PRNP of BARB cases (SEAC 88/4)

35. The Chair explained that the committee had been asked by Defra to comment on the findings of two studies comparing the sequences of the prion protein gene (PRNP) carried by BARB cases and healthy control animals, one carried out in Great Britain (GB) and the other in Northern Ireland (NI).
36. Dr Yvonne Boyd (Defra) provided the background to this issue. The committee was informed that since the detection of the first BARB case in an animal born in June 2000, there had been around 100 BARB cases reported in the UK. BARB cases had been identified by four surveillance streams: clinical suspects, casualty or emergency slaughtered animals, fallen stock, and cattle slaughtered under the over thirty month scheme (OTMS). The BARB cases were of concern because the aim of the implementation of the reinforced feed ban in August 1996 was to eliminate possible sources of BSE transmission in the UK.
37. The committee noted that the origins of infection in BARB cases were unknown. The most likely explanation for the BSE cases born after 1996 was that the cattle were still exposed to BSE contaminated feed. However, alternative sources for the BSE infection were also possible. At SEAC 85, possible origins of BARB cases were discussed. SEAC had recommended that PRNP sequencing of BARB cases be performed to address the issue of possible genetic predisposition to BSE, either spontaneously or by increased susceptibility to exposure from an exogenous source.
38. The committee was informed that any analysis of genetic predisposition for BARB cases was potentially difficult. Unlike human and sheep prion diseases, there is no experimental evidence for a genetic component in susceptibility to BSE in cattle. Even if there was a genetic predisposition to BSE in cattle, each breed of animal may have a different genetic association. It was noted that the BARB BSE cases have arisen in cattle of different breeds. Finally, the greater frequency of BARB cases immediately after the reinforced animal feed ban compared to the incidence of sCJD in humans and the steady decline in the numbers of BSE cases thereafter, indicates that many of the BARB cases are unlikely to be of spontaneous origin.

GB study

39. Dr Ginny Saunders (VLA) presented the findings of the Defra funded research on PRNP sequencing of BARB animals reported in GB. This research has been carried out at VLA in collaboration with the Roslin Institute. The aim was to sequence and compare the PRNP coding and promoter regions of all available BARB cases with cohort-matched controls, to ascertain if there was any association between DNA polymorphisms and disease.
40. The committee noted that the strategy for sequencing the 5 kb promoter region and 1 kb open reading frame (ORF) for the bovine PRNP locus is through generating overlapping polymerase chain reaction (PCR) products covering the entire length of the promoter region and ORF. The sequenced fragments were then to be compared with bovine PRNP sequences submitted to Genbank and published previously (Hills *et al*, 2001²). It is known that the bovine ORF normally has six octapeptide repeat regions, however in some individuals there is a 24 base pair deletion polymorphism resulting in only five octapeptide repeats.
41. The committee noted that the sequences of 73 of the 82 BARB cases available at the time of sequencing showed no significant differences to appropriate cohort-matched negative controls (n=63). The cohort-matched control animals were sourced to match the BARB cases to their age, sex, breed, and farm as far as possible.
42. The committee was informed that the study did not detect any new polymorphisms. Four previously documented mutations, at codon positions 23, 78, 133 and 192 of the ORF, had been found. The change at position 78 is the most common change noted in BARB cases and controls, apparent in 34% of BARB cases and in 32% of controls. All the changes were silent mutations which do not alter the amino acid sequence of PrP. The deletion of one octapeptide repeat was detected in 5% of BARB cases and 8% of controls. Statistical analyses showed no significant differences between BARB cases and controls for any of these polymorphisms.
43. The committee noted that the sequence of the same PRNP regions from the 20 most recently reported BARB cases had also been carried out and no significant differences in polymorphisms

² Hills *et al*. (2001) Complete genomic sequence of the bovine prion gene (PRNP) and polymorphism in its promoter region. *Anim. Genet.* 32, 231-232

compared with the initial 73 sequenced BARB cases was seen. These data have not yet been added to the original data for statistical analysis.

44. The 3 linked BARB cases reported in Pembrokeshire, Wales from the 2001/2002 birth cohort showed silent mutations in the octapeptide repeat regions at codon position 78. The case reported in 2001 had a homozygous change and the two cases reported in 2002 showed heterozygous changes. The ORFs of the two cohort-matched controls from this farm were sequenced. One had a wild type, and the other a homozygous five octapeptide repeat.
45. The committee noted that sequencing of the 5kb promoter region covering exon 1, intron 1 and exon 2 of around 170 animals was underway. This region had been defined as the promoter from previous studies in mice using expression of reporter genes.

NI study

46. Dr Alastair Douglas from the Department of Agriculture and Rural Development, Northern Ireland (DARDNI), presented the PRNP sequencing results of the BARB cases reported in NI. Thirteen of the 15 BARB cases reported to May 2000, randomly selected confirmed BSE cases born prior to August 1996 (n=13), and randomly selected age-matched negative control animals (n=26) were included in the study. Unlike the GB study, negative controls were not matched for breed, cohort or geographical distribution. All the groups included a variety of breeds and ages of animal. One BARB case was identified through the BSE clinical suspects programme, while the remaining cases were detected by the active surveillance programme.
47. The study investigated polymorphisms in the promoter and coding regions of PRNP as well as the coding region of the Doppel gene (a prion-like ancestral protein encoded by a neighbouring gene, PRND). In addition, four insertion-deletion (Indel) elements were analysed, including one which included the octapeptide repeat region.
48. A 1.5 kb section in the PRNP promoter region, which covers most of the previously reported polymorphisms, the PRNP coding regions and 0.5 kb (the entire coding region) of the Doppel gene, were sequenced and analysed. Polymorphisms were identified by comparison with the sequence of the study group and published

sequences submitted to Genbank (three for PRNP and 1 for Doppel).

49. Fourteen single nucleotide polymorphisms (SNPs) had been identified in the PRNP promoter region. One of these polymorphisms has not previously been reported. This new polymorphism was found in 40% of the study group. Five previously described SNPs were identified in the PRNP coding region, and two previously described SNPs in the Doppel coding region. With respect to the octapeptide repeats, the majority of the animals were homozygous for 6 repeats, with 3/52 animals heterozygous for 5 and 6 repeats. Statistical analysis showed no significant differences in the polymorphisms between the pre- and post-August 1996 BSE negative groups, between the post-August 1996 BSE positive and post-August 1996 BSE negative groups, and between the post-August 1996 BSE group and all BSE negative animals.
50. In the BARB animals, the only polymorphism significantly different from the control groups was a silent mutation at base pair 395 in the PRNP coding region, which does not alter the amino acid sequence of PrP protein. There was no evidence of a genetic mutation within the BARB group of BSE cases which could have contributed to disease status. Comparing disease status within the whole study group the BSE positive animals (born both before and after August 1996) showed a significant association of specific polymorphisms within the PRNP promoter region. One of these, the 23 base pair (bp) Indel polymorphism identified in these disease groups, has previously been reported as associated with BSE.
51. A member commented that promoter region polymorphisms could be functionally linked to BSE susceptibility through altering PrP expression levels. It was suggested that, although the 395 bp polymorphism found in the BARB cases is a silent mutation, it is also possible it might affect the level of PrP expression. As the two studies reported here used different criteria to select control groups the results are difficult to combine. However, there appeared to be consensus that the 23 bp Indel is significantly enriched in the BSE population in NI.
52. A member asked whether, as the BARB cases are mostly reported in Friesian Holstein animals, there is any evidence that different PRNP polymorphisms may affect different breeds of animals. Members were informed that studies are ongoing to analyse breed susceptibility to BSE.

53. The committee considered that:
- there was some accumulating evidence of a linkage between a specific PRNP promoter polymorphism and susceptibility to BSE
 - although the number of BARB cases in these studies is small (n=108), the results indicated that there is no linkage between any PRNP polymorphism and the BARB BSE cases, specifically
 - there is no genetic (or other reason) to suppose that BARB BSE cases are any different than pre-August 1996 cases.
 - the committee asked for a copy of the DARDNI report on the NI studies for reference.

Response to the SEAC ad hoc Epidemiology Subgroup on UK BARB cases

54. At SEAC 87, the committee received a report from the SEAC *ad hoc* Epidemiology Subgroup on UK BARB cases which was advising Defra on the design of a case control study to identify possible causes of BARB BSE cases. SEAC had endorsed the Subgroup's recommendations for further analysis of the results using different groups of controls, and for prospective evaluation of animal feed use and supply routes and the potential for cross-contamination of feeds. Professor John Wilesmith informed members of work undertaken by Defra following the Subgroup's recommendations.
55. The first recommendation had been to restrict the case control study to clinical cases and suspects only. Following this, 27 BARB cases and 367 controls identified by passive surveillance were identified. Bivariate statistical analyses indicated a similar trend as in the previous study, using BARB cases ascertained through both active and passive surveillance. Proprietary feeds and higher numbers of previous cases on farm appeared protective against BARB occurrence, although this finding was not statistically significant. Apart from age at onset, multivariable analysis did not identify any statistically significant risk or protective factor. Members were informed that no further insight into BARB cases could be gained from this approach.
56. The second recommendation had been to perform a case control study using a random sample of controls from the Cattle Tracing System (CTS). Defra had decided against this approach as an unbiased sample was difficult to achieve. The CTS had not yet been established on a herd basis and the number of risk factors

that could be examined using the available data was limited. Professor Wilesmith indicated that an alternative approach would be to conduct an ordinal logistic regression, using the cattle holding as the unit of interest, and the agricultural census to draw the controls. This approach, together with a spatial component, would enable analysis of the distribution of cases on a herd basis in the three phases of the BSE epidemic, and for BARB cases.

57. Professor Wilesmith informed members of apparent short lived risks for various BARB birth cohorts in specific geographical areas, with the caveat that the numbers of BARB cases per birth cohort are small. Analysis of BSE cases from the 1996/1997 cohort indicated a statistically significant higher incidence in South East England, which has a low density of dairy herds. The higher incidence of BARB cases was not apparent in SE England in the 1997/1998 birth cohort, but was instead focused on West Wales, where a triplet of cases and also paired cases had occurred. In the 1998/1999 cohort the higher incidence in West Wales was not evident, however triplet cases, born within 14 days of each other, had occurred in a Wiltshire herd. Members were informed that numbers of BARB cases per birth cohort are too small for the combination of spatial and temporal analyses recommended by the Subgroup, however a spatial analysis is in progress.
58. The third Subgroup recommendation was to perform an evaluation of animal feed use and supply routes. Professor Wilesmith indicated that Defra was continuing to follow up the feed history of individual BARB cases. Defra is reviewing the potential for cross contamination of feed with respect to regulatory guidelines and compliance. However, since the European feed ban of 2001, little TSE-contaminated feed should be in circulation.
59. BARB BSE prevalence estimates are based on a back calculation from cases reported and detected by 31 March 2005, for the 1996/1997 birth cohort through to the 2000/2001 cohort. Although the numbers of BARB cases per cohort are small, the estimates show evidence of a statistically significant decline in prevalence between the 1996/1997 cohort (130 infected cattle per million, 95% CI 80-180) and later cohorts. The prevalence in the 1998/99 and 1999/2000 cohorts was identical on current data (40 infected cattle per million, 95% CI 20-70). No BARB cases have been detected to date in the 2000/2001 cohort. Members were informed that if the West Wales triplet of BARB cases from the

2001/2002 birth cohort is included this gives a higher BARB prevalence, although the upper confidence limit is very high.

60. Members noted that the feed exposure for the triplet cases related to feed manufactured in 1998, not in 2001 or 2002. The committee also noted that the risk of a herd developing a BARB case has shown a decline of one third since BARB cases were first detected.

Independent Review of the cause(s) of BARB cases (SEAC 88/5)

61. The Chair thanked Professor William Hill (University of Edinburgh) for his comprehensive report, commissioned by Defra, describing his independent review into the cause of BARB cases which had been made available to SEAC members. Professor Hill was asked to provide an overview of his report which will be published in due course.
62. Professor Hill informed the committee that although he assessed alternative hypotheses as to the causative agent of BARBs, he considered there was no evidence to suggest that BSE in BARB cases was different to that observed pre 1996.
63. Professor Hill thought it unlikely that the majority of the BARB cases arose spontaneously, commenting that the USA had had only one recent case, yet the US has a dairy population of a type similar to that of the UK. He could find no evidence to support the idea that genetic variation in cattle had important effects on susceptibility to this disease either by infection or as spontaneous cases. There was no evidence that BARB BSE cases are being transmitted in a different way to previous cases of BSE.
64. Members noted that there might be a low level of spontaneous cases, which has implications for the total eradication of BARB cases.
65. The Chair recommended that the GB and Northern Ireland genotyping data should be combined if possible for statistical analysis, but assuming that there was no significant link between susceptibility and polymorphisms at the PRNP and PRND loci, it may not be essential to genotype all new BARB cases, with the exception of potential atypical cases. It was recommended that rapid access to sequencing be facilitated as this appears to have been a limiting factor in the past.

66. Professor Hill had evaluated the epidemiological evidence and found no evidence of maternal or lateral transmission. The majority of BARB BSE cases were born from dams that were not affected, and BARB cases had been detected on farms with no prior BSE. The committee agreed with this conclusion.
67. Professor Hill stated that limited infection via environmental or other non-food borne contamination cannot be excluded. However, the likelihood is low given current knowledge of the disease in cattle and the absence of other evidence. The persistence of the infective agent in the environment, for example on the land or in water sources, is not known. It might be worth considering investigating prior cases of BSE in neighbouring farms to BARBs as a route to checking environmental contamination.
68. FATEPriDE, a major EU funded study examining environmental risk factors that affect the development of prion diseases such as BSE, is currently unable to study the link between some environmental variables and BSE (including BARBs) incidence as they have not obtained the necessary data from Defra. Professor Hill recommended that this study should be facilitated and the committee concurred.
69. Feed borne infection is generally considered to be the major route of BSE infection for animals born prior to 1996. A recent study showed that very low doses of infected neural tissue can cause BSE. Professor Hill considered that contamination of feed remained the most likely cause of BARB cases. No other reasonable hypothesis fits the available data. He noted that pairs and triplets of BARB cases were consistent with this view. In addition, epidemiological evidence, low levels of disease and the change in geographical distribution from East Anglia (for pre-1996 BSE) to a more widespread distribution in the UK (BARB cases) suggests that feed is an important factor. Although the incidence of BARB cases and BSE is falling in the UK and Europe, and most of the controls to reduce the number of cases are already in place, it was possible that residual contaminated feed may still be present on some farms. Feed may have been imported from abroad prior to other countries adopting the ban on MBM. The elimination of feed borne sources is crucial to the elimination of BARB cases.
70. A member was concerned about the balance between passive and active surveillance in relation to their relative contribution to the epidemiological study. Another member was concerned

whether the surveillance methods identified all cases of BARBs. Professor Wilesmith reassured the committee that information was collected on all suspect cases in the first instance, and then the cases that were confirmed clinically as BARBs were assessed so it is unlikely that cases were being missed.

71. A member was informed that there was no evidence to suggest that BARB cases were atypical forms of BSE. Members agreed that active and passive surveillance should be maintained worldwide to identify any potential strain variation of BSE that might give rise to atypical cases.
72. Determining the control measures necessary was problematic as it was difficult to detect the very low levels of contamination in feed that can cause disease. Members agreed that control measures should be based on transmission of the infectious agent by feed, however implementation of control measures was not within SEAC's remit. Members agreed that research capacity should be maintained to establish the feed-borne cause of BARB cases.
73. A member queried the wording of the recommendations in the general conclusions of the report regarding existing controls. Barrowman noted that the wording would be changed to take account of the potential change to the OTMR.
74. It was pointed out by members that care needed to be taken not to confuse the amount of PrP^{Sc} protein with levels of infectivity because of uncertainty regarding the precise nature of the infectious agent. It was suggested that the wording of the finalised report should take account of this point.
75. Members recommended relevant research should be maintained to determine if there is more than one cause of BARBs and if the agent causing BARBs is the same as BSE.
76. The Chair reminded the committee that this was not a SEAC report but one commissioned by Defra and that SEAC had simply been given an opportunity to comment prior to publication.
77. In conclusion, the committee welcomed the report and agreed with the main conclusions. Suggestions were made where modifications might be helpful for clarity and completeness.

ITEM 7 – SEAC EPIDEMIOLOGY SUBGROUP REPORT (SEAC 88/6)

78. Professor Graham Medley (Chair of SEAC Epidemiology Subgroup) updated the committee on the Subgroup's first meeting on 11th May 2005 to discuss the nature and future profile of the vCJD epidemic. The Subgroup had discussed the importance of surveillance methods such as the National Anonymous Tonsil Archive (NATA), as well as others, to allow direct ascertainment of current vCJD infection prevalence and the influence of genotype and age on infection prevalence. Discussions had also focused on modelling work which might be used to assess how infection prevalence might change and the interaction of potential routes of secondary transmission. Work was continuing, with a second meeting scheduled in September 2005.
79. Members asked about the progress of NATA. The Subgroup Chair responded that the study was on track. Most of the target hospitals had been recruited, a protocol to collect and store the tissue was in place, and samples were being collected. Testing of samples had not begun and was still subject to ethical consideration. The SEAC Chair added that the Subgroup could make recommendations on ethical issues to SEAC in its final report on the testing of samples from NATA.

ITEM 8 – ANY OTHER BUSINESS

80. The Chair drew members' attention to the Update on the National Scrapie Plan (paper SEAC 88/7), provided for information.