

# RESPONSE TO SEAC RECOMMENDATIONS ON THE DIFFERENTIAL DIAGNOSIS OF BSE IN CATTLE

## SUMMARY

SEAC members have noted their interest in the differential diagnosis, variations in phenotype and strain stability of BSE in cattle in view of phenotypic differences recorded in TSE infections in humans and in sheep.

From the clinical suspect cases in cattle that are reported each year there is a proportion that are subsequently not confirmed as positive BSE cases. The aetiology of these non-confirmed suspect cases includes a very long list of potential conditions and 40-60% of cases show no significant neuropathological lesions. Given these two observations and the limitations of veterinary clinical neurology Defra does not see a proportionate value in attempting to arrive at a definitive diagnosis for all non-BSE suspect cases. Notwithstanding this view, Defra would require the more detailed investigation of cases which may give rise to a suspicion of clinical or pathological BSE variant, when sufficient appropriate material is available. Efforts will also be maintained to optimise the clinical screening of suspect cases to reduce the number of unconfirmed cases.

Work carried out by Defra (and previously by MAFF) on surveillance and testing of suspect cases during the course of the BSE epidemic in cattle has not found evidence for strain variation or change in the neuropathological or molecular characteristics of the disease. There is scope for applying more recently developed methods retrospectively to samples collected during the epidemic.

The disease phenotype of BSE is defined on the basis of clinical signs and post-mortem neuropathology. The known phenotypic expression of all TSE diseases both clinically and pathologically involves the central nervous system. Defra therefore consider that it is appropriate to use clinical and case history together with post-mortem screening of changes in the brain as indicators for phenotypic variation of BSE in cattle. Phenotype discrimination can also be monitored by variations in PrP<sup>res</sup> molecular profile using advances in immunoblotting methods that have been developed in recent years. The focus of resources to these areas of activity is considered to be proportionate and appropriate for the monitoring of possible changes in BSE in cattle.

It is recognised that with the falling numbers of clinical BSE cases the opportunities for prospective study will be limited. The remaining infected animals are principally detected through surveillance testing of cattle at slaughter. There is very little sample material available from these cases and it is often of variable condition. Furthermore any clinical investigation is retrospective and possibly influenced by virtue of a known diagnosis.

This paper outlines the approaches that have been taken by Defra (MAFF) during the course of the BSE epidemic in cattle.

# **Defra response to SEAC recommendations on the differential diagnosis of BSE in cattle**

## **ISSUE**

1. SEAC members have put the question of what work is being done to enable the detection of an altered form of BSE or another unknown or associated disease in cattle. This has been recorded in the SEAC minutes on two recent occasions:

- 1.1. SEAC 84 (28 September 2004). Item 2. open minutes. Paragraph 10

“10. A member noted that a reduction in the number of confirmed clinical cases of BSE had been observed and asked whether differential diagnostic procedures were in place to allow the detection of an altered form of BSE or another unknown disease. [...] Members considered that research on methods to allow differential diagnosis of clinical cases of BSE was important particularly in view of evidence on the phenotypic differences in infection in humans and sheep.”

- 1.2. SEAC 85 (30 November 2004). Minutes. Paragraph 8

“8. At the meeting on 28th September 2004, members noted that there had been a number of cases of animals with clinical signs consistent with BSE that were not confirmed as BSE using diagnostic tests. In view of these cases and the phenotypic differences of TSE infection in sheep and humans, members had suggested that research on methods to allow differential diagnosis of clinical cases of BSE was important. One member asked whether any progress had been made relating to this issue. The Chair agreed to pursue the matter with Defra.”

## **PRESENT POSITION**

2. In considering the concerns of SEAC, the issue can be separated into several component questions:

- 2.1. Can the diagnosis by clinical means of suspect BSE cases be improved?

- 2.2. Has BSE changed in cattle, so that a new form may be occurring that may not be detected by current methods?

- 2.3. Are other previously unknown or unrecognised diseases related to TSEs appearing in UK cattle?

3. These questions can in turn be considered in several sections and addressed individually, although there is inter-linkage between them:

- 3.1. Differential diagnosis by clinical means in live cattle suspected of having BSE

- 3.2. Differential diagnosis by means of diagnostic tests in live cattle suspected of having BSE

- 3.3. The examination of post-mortem material from BSE clinical cases confirmed positive

- 3.4. The examination of post-mortem material from suspect BSE cases not confirmed positive

- 3.5. Methods for the differentiation of new variants or strains of BSE in cattle.

## Differential diagnosis of BSE by clinical means in live cattle

4. Defra, the Devolved Administrations, the State Veterinary Service (SVS) and the Veterinary Laboratories Agency (VLA) are responsible for the identification, diagnosis and confirmation of BSE in cattle. The VLA at Weybridge is the National Reference Laboratory. This process has been carried out since BSE came under statutory control in June 1988 and at its simplest involves several steps:
  - 4.1. Suspect cases based on clinical signs and history are reported to the SVS by a veterinary surgeon or stock-holder.
  - 4.2. The case is examined by a State Veterinary Officer and statutory procedures are put in place if the suspicion is confirmed. Details of the case are recorded on a BSE1 form which collects information for epidemiological analysis.
  - 4.3. Suspect cases are euthanased and post-mortemed by the VLA using approved procedures by trained personnel. Defined parts of the central nervous system mainly focussed on the brain stem (obex) are removed for defined diagnostic procedures. Throughout the epidemic a number of longitudinal studies have removed and examined multiple brain areas from a small population of suspects. As the epidemic has declined the procedures have changed. Whole brain is currently sampled from every clinical suspect case, multiple brain areas have been taken where possible from suspects born in 1996 or later.
  - 4.4. Diagnosis is confirmed by a suite of pathology investigations and physico-chemical tests for PrP<sup>d</sup> at the VLA Weybridge.
5. Fundamental to this procedure is the accuracy with which BSE can be identified in the live animal by the stock-holder, the practicing veterinarian and the State Veterinary Officers.
6. Defra, the Devolved Administrations, the SVS and the VLA have gone to considerable lengths to study this new disease in cattle, to define and publicise its clinical characteristics and to ensure that information and training is available to veterinarians. This continuing professional development has recently been augmented with the release of a DVD covering the clinical disease in detail. Video clips of the clinical appearance of the disease are also presented on the VLA website and can be seen across the world.
7. There are a large number of disease states, both infectious and non-infectious, that can occur in cattle in which the clinical appearance can overlap with that of BSE. The differentiation of these conditions can be difficult or impossible even to experienced clinicians. In addition, all the recognised BSE clinical signs are not necessarily always expressed in affected cattle<sup>1</sup>. These difficulties are reflected in the number of suspect cases of BSE which are not confirmed as positive after post-mortem.
8. Clearly Defra wish to significantly reduce or eliminate the number of clinical suspect cases that do not go on to be confirmed as BSE. At the same time it is important that BSE cases should not go undetected. To address this challenge Defra is giving

consideration to the means of maintaining optimum clinical evaluation of suspect cases, particularly when BSE suspect cases are no longer being confirmed.

9. Given the very large number of conditions which might resemble BSE clinically to varying degrees<sup>2</sup>, the Department does not see the proportionate value of attempting to arrive at a definitive diagnosis for all suspects not confirmed as BSE (Annex A). The science of clinical neurology in cattle is not well developed, a significant proportion of cases are likely to remain as of unknown aetiology, and the investment in resources would be considerable. This view has been expressed by Defra officials to SEAC at recent meetings.
10. Suspect cases will continue to occur and the use of methods to support clinical diagnosis, e.g. to exclude metabolic disorders or conditions of non-CNS aetiology, can be reinforced. Where BSE cannot be confirmed or ruled out under a restriction order the case can be re-evaluated regularly until BSE can be confirmed or excluded on clinical grounds. During this time the stock-owner is encouraged to pursue other possible causes of disease with their private veterinary surgeon. It can be anticipated however that there will continue to be a small number of suspect cases where CNS involvement is present. In the absence of clinical changes which are definitive and pathognomonic for BSE the final diagnosis of BSE will remain with the post-mortem procedures described below.
11. The phenotypic variation in disease that has been the subject of reports for TSEs of humans and sheep still involves clinical changes resulting from CNS pathology. The screening of cattle for variations in the phenotype of BSE or the emergence of another similar disease can be achieved through the appropriate post-mortem examination of suspect cases in which CNS involvement could not be excluded on clinical examination.
12. Other than the early involvement of intestinal Peyer's patches there is currently no evidence that the pathology of clinical BSE in cattle occurs without CNS involvement. It is therefore considered appropriate to use post-mortem screening of pathological changes in the brain and physico-chemical tests as indicators for phenotypic variation of the disease. The focus of resources on CNS pathology and immunochemical testing is considered to be appropriate and proportionate.

### **Differential diagnosis by means of diagnostic tests in live cattle suspected of having BSE**

13. Since the early days of BSE and vCJD those engaged in disease surveillance and the Government funders of TSE research have identified the need for a live animal test as a high priority and significant investment has been made in this area. The call has extended to all scientific disciplines on a global scale.
14. This investment has not so far led to a viable chemico-physical or immunological test that can be used either clinically or pre-clinically for the diagnosis of BSE in the live animal. Given our present knowledge of the disease this is not unexpected. There is no overt immune response mounted against the agent as in most other transmissible diseases and there is very little known extension of the pathology outside the CNS in cattle. Although it is theoretically possible to search for products of neurodegeneration

in the circulation, this has not been rewarding and is not likely to be specific. Protease-resistant PrP, which is the marker characteristically associated with the disease, has not been demonstrated to date in circulating body fluids accessible for testing.

15. The concept of using the measurement of autonomic nerve functions which might be affected by pathological changes in the brain stem and spinal tracts has been postulated. Alterations in the patterns controlled by autonomic nerves such as rumen-reticular cycles, cardiac cycles, and baroreceptors have been studied. No aid to diagnosis based on this concept is yet available. Obtaining readings of this type in infected animals which are known to exhibit hyperaesthesia and aggressiveness is considered to present safety and practical difficulties under field and laboratory conditions. Any detectable alterations would be indicative of brain stem pathology but not specifically of BSE.

### **The examination of post-mortem material from BSE clinical cases confirmed positive**

16. The diagnosis of BSE in cattle is confirmed by the VLA on samples taken from suspect cases at post-mortem. The obex of the brain is the target site for diagnosis and there is a standard protocol for sampling at post-mortem. Whole brain is also archived from many of the cases identified with clinical disease through passive surveillance.
17. There are currently twelve EU approved rapid tests for the confirmation of BSE in cattle (Annex B), all of which are based on the detection of protease resistant PrP. Suspect samples are examined by the VLA using two of these approved tests.
18. In addition to the rapid tests which are now in use, the samples are also examined by histopathology and immunohistochemistry which were developed to a high level of proficiency before the introduction of the rapid tests. Immunohistochemistry methods have been applied to all suspect cases born since August 1996.
19. A combination of positive results from this suite of tests is used for statutory BSE confirmation in the UK.
20. In addition to the cases which are investigated as a result of notification of suspects (passive surveillance) other positive cases are identified through active surveillance of cattle over 30 months of age at slaughter. These cases are identified from the use of EU approved screening and confirmatory tests on a sample of the obex collected according to a standard procedure after slaughter. Although providing a positive/negative result these samples are very limited and do not provide the opportunity for clinical or full biological investigation of the case. The significance of this for phenotype determination is discussed below.

### **The examination of post-mortem material from suspect BSE cases not confirmed positive**

21. The numbers of BSE suspect cases that are not confirmed as BSE following histopathological examination have followed a variable range throughout the BSE epidemic, but with the declining number of positive BSE cases the unconfirmed cases have assumed a progressively higher percentage of the total suspect cases. The

numbers examined through passive surveillance during the epidemic are listed in Annex C.

22. A number of studies funded by Defra involving the neuropathological analysis of the brains from unconfirmed BSE suspects report that a high proportion of the total samples examined show no significant pathological lesions (53.5%<sup>3</sup>, 40.1%<sup>4</sup>, 61-61.7%<sup>5</sup>) which could be used as the basis for differential diagnosis.
23. From the non-BSE cases in which pathological changes were found several previously described disorders of the central nervous system were observed, including some conditions that had not been previously reported in Britain.
24. Additional histopathological features, including vacuolation of the white matter and mineralisation of the blood vessels were observed in some BSE negative cases. These features were later also observed in the brains of symptomless aged (7 year old) cattle<sup>6</sup> and could be considered to be the result of ageing.

#### **Methods for the differentiation of new variants or strains of BSE in cattle**

25. Monitoring BSE in cattle for the existence of variant forms of the disease is of practical concern because of the different effects that variants could have on the control and eradication of the disease. Of particular importance would be variants that result in change of the mode of transmission or the susceptible host range.
26. Disease phenotype can be defined on the basis of biological characteristics in the affected host(s), the reference set of features being determined in the clinical stages when the disease is fully expressed. In the case of TSEs these characteristics include:
  - Species, population, gender and ages affected
  - Clinical signs
  - Histopathological changes in the CNS and PrP<sup>d</sup> distribution
  - Tissue distribution of the agent
  - Transmissibility within host species
  - Genomic differences in susceptibility
  - Transmissibility to other species
27. The initial description of BSE in cattle<sup>7</sup> was based on case histories, clinical signs, neurohistological changes and the presence of PrP<sup>d</sup> aggregates or fibrils in brain extracts<sup>8</sup>. This reflects the situation for human forms of TSE and sheep scrapie where neuropathology is used in the primary definition of phenotype. Subsequently the distribution of PrP<sup>d</sup> in the brain shown by immunohistochemistry has offered a more sensitive and specific additional approach to phenotype characterisation<sup>8</sup>.
28. Veterinary pathologists examining cattle brains early in the BSE epidemic in the UK reported that the pathological lesion profile and distribution observed is stereotypic which was suggestive that only one strain of the agent is present<sup>8 9 10 11</sup>. Studies were therefore put in place to examine the lesion profiles in brain from suspect cases during the course of the BSE epidemic to determine whether they remained constant, consistent with the epidemic continuing to be sustained by a single strain of BSE agent without the emergence of new variants exhibiting an altered biological phenotype profile. From the examinations reported to date a stable lesion profile has been

observed in confirmed BSE cases<sup>4 5</sup>. This has provided a baseline signature of the natural disease phenotype as a control for future studies.

29. Studies on cases of BSE in cattle outside GB have reported pathological features consistent with the GB baseline phenotype. These include reports from Switzerland<sup>12 13</sup>, Portugal<sup>14</sup>, Spain<sup>15</sup>, Italy<sup>16</sup> and France<sup>17</sup>. As OIE and EU reference laboratory the VLA has as yet unpublished results of initial cases from USA, Canada, Italy, Denmark and Japan of pathological features consistent with the GB pathology phenotype.
30. The definition of disease phenotype in BSE affected cattle was augmented by the quantification of selected biological characteristics following transmission to a panel of inbred mice. This standardised procedure based on incubation period and profile of brain lesions had previously been developed as a research tool for discriminating scrapie strains in sheep. This method demonstrated a consistent signature for isolates of BSE from cattle which was different from that of scrapie isolates<sup>18 19 20</sup>.
31. During the course of the BSE epidemic in cattle the in vitro molecular characterisation of abnormal or disease-related PrP<sup>res</sup> has evolved with improvements in separation techniques and the development of antibodies. Differential glycosylation and partial proteolysis of PrP<sup>d</sup> extracted from brain and separated by SDS-polyacrylamide gel electrophoresis and Western Blotting can give patterns of immuno-reactive bands which are interpreted as a signature of the PrP<sup>d</sup> type or strain. Molecular analysis by these means has become a useful adjunct to TSE phenotyping.
32. Clinically suspect cases of BSE in cattle born since January 1996 and identified by passive surveillance in GB have, in addition to case and clinical histories, been examined consistently with three confirmatory methods – CNS histology, PrP<sup>d</sup> immunohistochemistry and Western Blotting. No significant deviations from the overall phenotype stereotype have been reported from these examinations. Prior to this considerable effort was made to establish and monitor the phenotype of BSE based on the vacuolar pathology of the brain. Since the introduction of PrP differentiation methods there have only been limited studies of retrospective material to consolidate knowledge of the BSE phenotype. The full phenotypic characterisation of each and every BSE case (as conducted for CJD and vCJD cases in humans) has in the past not been possible due to the magnitude of the number of cases and the availability of the time of expert neuropathologists engaged in the confirmation of positive cases.
33. Tests revealing a small number of cattle with molecular features atypical of BSE have been reported from Japan<sup>21</sup>, Italy<sup>22</sup> and France<sup>23</sup>. These cases have been found in active surveillance programmes through the application of rapid immunological tests on samples of brain and no clinical or case history is available. The atypical features are confined to molecular profiles of PrP<sup>d</sup> on Western Blotting for all three cases from France and one of the two cases from Japan (the other has a profile typical of BSE). Studies in France are ongoing and the publication of more results is expected. The two Italian cases have atypical PrP<sup>d</sup> electrophoretic profiles which are supported by differences in the pattern of brain distribution of PrP<sup>d</sup> observed by immunohistochemistry\*.

34. Information to provide a composite phenotype of BSE has evolved since the first recognition of the disease in cattle in 1986. Concern over the present or future existence of variants of the disease requires that a baseline phenotype is described and that the significance of variations of one or more components should be assessed against this baseline profile. Of particular importance for disease control are factors which reflect a change in behaviour of the disease such as variations in transmissibility or host range. A full understanding of the molecular and biological aetiology of prion diseases may eventually supercede the definition of phenotype based on biological characteristics.
35. For operational purposes Defra is advised that the evaluation of possible variants of BSE and their significance should be done by considering their relevance to the full range of biological components in the baseline phenotype. This approach should be used until such time that the body of scientific information provides acceptable evidence that one or other variable is identifiable as a statistically reliable indicator of change in the disease. The identification of individual components at variance with the baseline should lead to increased investigation on the cases of origin.
36. The increased use of *in vitro* rapid immunodiagnostic tests has the advantage of providing information on the status of individual animals and the prevalence of the disease in populations. Tests using molecular separation techniques also provide comparative information on the nature of PrP<sup>d</sup> isolated from infected individuals. In some cases across the spectrum of TSE diseases there is an apparent correlation between the type of PrP<sup>d</sup> consistently isolated and the biological phenotype of the disease with which it is associated. Current information on this relationship is not sufficiently comprehensive or validated to support the concept that molecular PrP<sup>d</sup> types a priori reflect different biological phenotypes of disease expression. The lack of a one-to-one correlation of disease phenotype with PrP<sup>d</sup> profile means that this molecular signature cannot as yet by itself be used to define disease phenotype.
37. The effectiveness of control measures for BSE in cattle has resulted in a dwindling number of live clinical cases being available for biological phenotype confirmation. Most of the remaining infected animals are being detected by active surveillance of cattle at slaughter. Only small amounts of material (obex) are available from these animals. The only clinical history, if any, is retrospective and the large majority will be incubating the disease. The monitoring of fallen stock or casualty cattle for BSE cases is one possible source of future material for monitoring biological phenotype of the disease.
38. Efforts are being made to maximise the gathering of information from the remaining cases of BSE occurring in GB to detect variance that has possible implications for infection with a prion disease agent other than that defined for BSE. Whenever suitable material is available whole brain will be collected for examination for pathological changes and immunochemistry in other areas of the brain. Information on the behaviour of the disease will also be derived from continuing epidemiological studies of the epidemic which would indicate if the nature of the disease is changing. Apart from these forward investigations there remains the opportunity to carry out retrospective examination of archived material to further investigate the variability of disease phenotype during the epidemic.

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\* The Italian cases were considered at SEAC 81 with the conclusion:

“In view of the limited data and scientific uncertainties, SEAC was cautious about the extent to which these results could be interpreted. The Committee agreed that atypical phenotypic differences alone did not constitute sufficient evidence to demonstrate the identification of a new strain of BSE. Further research such as mouse-strain typing were critical to this work before any interpretation or claims could be verified.”

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<sup>4</sup> Jeffrey, M. (1992) A neuropathological survey of brains submitted under the Bovine Spongiform Encephalopathy Orders in Scotland *The Veterinary Record* **131**, 332-337

<sup>5</sup> Simmons, M.M. *et al.* (1996) The BSE epidemic: consistencies of neurohistological findings in annual samples of clinical suspects *Cattle Practice* **4**, 361-364

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<sup>18</sup> Fraser, *et al.* (1988) Transmission of bovine spongiform encephalopathy to mice. *Veterinary Record* **123**, 472

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