

ALTERNATIVE HYPOTHESIS – AN AUTOIMMUNE ORIGIN FOR TSE**Issue**

1. The Chief Medical Officer for England has asked SEAC to consider reassessing the plausibility of an alternative hypothesis for the origin of transmissible spongiform encephalopathies proposed by Professor Alan Ebringer (King's College London).

Background

2. A paper by Ebringer *et al.* (2007)¹ explaining the basis of the hypothesis in detail is provided at Annex 1. Briefly, the authors propose that, following oral exposure to the common soil bacterium *Acinetobacter*, antibodies to components of the bacterium may be produced by the host that can cross-react with components in the mammalian central nervous system (myelin and neurofilaments). It is suggested that this occurs because of structural similarities between myelin, neurofilaments and components of *Acinetobacter*. Such cross-reactivity could stimulate an autoimmune response in the host that results in neurodegeneration.
3. At SEAC 72 (February 2002), the committee discussed this hypothesis with Professor Ebringer and reviewed two studies which looked at levels of antibodies to *Acinetobacter*, and to bovine myelin and neurofilaments, in Bovine Spongiform Encephalopathy (BSE) cases. In the first study, of 29 confirmed BSE cases, 18 suspect cases later found not to be BSE and 58 healthy animals, higher levels of antibodies to *Acinetobacter calcoaceticus* (but not to two other types of non-*Acinetobacter* bacteria) were found in sera from the BSE group compared with the BSE-negative and healthy control groups ($p < 0.001$). Levels of antibodies to bovine myelin ($p < 0.001$) and bovine neurofilaments ($p < 0.001$) were also higher in the BSE group compared with the control groups². In the second study, higher levels of antibodies to *Acinetobacter radioresistens* (but not to six other types of non-*Acinetobacter*

¹ Ebringer *et al.* (2007) From rabies to transmissible spongiform encephalopathies: an immune-mediated microbial trigger involving molecular mimicry could be the answer. *Med. Hypothesis*. 68, 113-124.

² Tiwana *et al.* (1999) Autoantibodies to brain components and antibodies to *Acinetobacter calcoaceticus* are present in Bovine Spongiform Encephalopathy. *Infection & Immunity*. 67, 6591-6595.

bacteria) were found in sera of a group of 128 confirmed BSE cases compared with a group of 63 BSE-negative and a group of 64 healthy animals ($p < 0.0001$). Levels of antibodies to bovine myelin ($p < 0.0001$) and bovine neurofilaments ($p < 0.0001$) were also higher in the BSE group compared with the control groups³.

4. The minutes of the discussion at SEAC 72 are reproduced at Annex 2. SEAC concluded that “... *Professor Ebringer supported his theory by using a rather selective and limited choice of publications, and had disregarded much of the published literature. ... the theory was not a good postulate for the origin of BSE and it did not seriously challenge the prion hypothesis.*”
5. Since then, further research by Professor Ebringer has shown that higher levels of anti-*Acinetobacter* antibodies ($p < 0.001$) and myelin basic protein antibodies ($p < 0.0001$) were present in the sera from two sporadic Creutzfeldt-Jakob Disease (sCJD) patients compared with 29 healthy controls⁴ and from seven sCJD patients compared with an unspecified number of controls (p value not given)¹.

Advice sought from the committee

6. Members are asked to consider firstly, whether they are aware of any new data, beyond that referred to in paragraph 5 above, that would support Professor Ebringer’s hypothesis and secondly, whether the committee should discuss the hypothesis in detail with Professor Ebringer at a subsequent meeting.

³ Wilson *et al.* (2003) Antibodies to *Acinetobacter* bacteria and bovine brain peptides, measured in Bovine Spongiform Encephalopathy (BSE) in an attempt to develop an ante-mortem test. *J. Clin. Lab. Immunol.* 52, 23-40.

⁴ Ebringer *et al.* (2004) Multiple Sclerosis, sporadic Creutzfeldt-Jakob Disease and Bovine Spongiform Encephalopathy: are they autoimmune diseases evoked by *Acinetobacter* microbes showing molecular mimicry to brain antigens? *J. Nutr. Environ. Med.* 14, 293-302.

ANNEX 1

Ebringer *et al.* (2007) From rabies to transmissible spongiform encephalopathies: an immune-mediated microbial trigger involving molecular mimicry could be the answer. *Med. Hypothesis*. 68, 113-124.

ANNEX 2

SPONGIFORM ENCEPHALOPATHY ADVISORY COMMITTEE
Minutes of the 72nd meeting held on 06 February 2002 at DEFRA,
Conference Room A, Whitehall Place West, London

- Members:**
- Professor P Smith (Chairman)**
 - Professor A Aguzzi**
 - Professor R Anderson**
 - Professor C Bostock**
 - Professor R Carrell**
 - Professor J Collinge**
 - Dr D Cunningham**
 - Professor J Ironside**
 - Professor H Kimbell**
 - Mr P Jinman**
 - Professor C Masters**
 - Professor I McConnell**
 - Dr J Safar**
- Technical Advisors:**
- Mr P Soul (DEFRA)**
 - Dr H Gates (DEFRA)**
 - Dr J Stephenson (DH)**
 - Dr N Connor (DH)**
 - Dr S Dixon (FSA)**
 - Ms A Conroy (FSA)**
- Observers:**
- Dr A Douglas (DARDNI)**
 - Dr M Simmons (NAW)**
 - Dr J Neilson (HSE)**
 - Dr N Coulson (DEFRA)**
 - Dr M Donaghy (SEHD)**
 - Dr A Allman (BBRSC)**
 - Dr M Pitman (MRC)**
 - Dr D Matthews (VLA)**
- Secretaries:**
- Dr M Bailey (DEFRA)**
 - Dr R Jecock (DH)**
 - Mr A Harvey (FSA- Afternoon only)**
- Secretariat:**
- Dr L Harbron (DEFRA)**
 - Mr H Needham (DEFRA)**
 - Dr A Leigh (DH)**
 - Mr M Hall (DH)**
 - Dr I Hill (FSA)**

Also in attendance: Professor D King (Chief Scientist- Morning only)

Mrs J Griffin (Morning only)
Professor A Ebringer (Item 5 only)
Professor D Davies (Item 5 only)
Mr J Cleland (Item 5 only)
Dr K Meldrum (Item 5 only)
Dr R Pechlaner (Item 5 only)
Dr J Crowther (Item 5 only)
Dr L Hughes (Item 5 only)
Dr C Wilson (Item 5 only)
Dr H Davies (Item 5 only)
Dr D Brennan (Item 5 only)
Dr M Kibblewhite (Environment A.- Item 6 only)
Dr P Crook (Environment A. - Item 6 only)
Mr M Purcell (DNV Consulting- Item 6 only)
Mr J Spouge (DNV Consulting- Item 6 Only)

Item 5- BSE: Autoimmune Theory (SEAC 72/4)

- 5.1 The Chair welcomed Professor Ebringer and invited guests to the meeting. Professor Ebringer gave a presentation outlining both the autoimmune hypothesis for the cause of BSE and the diagnostic test that had been developed on the basis of the proposed aetiology.
- 5.2 Professor Ebringer outlined the central strands of his autoimmune hypothesis. Autoimmune diseases are characterised by the presence of antibodies which cause self-inflicted tissue damage to an organism. The generation of such antibodies is thought to be due to close structural similarities between foreign proteins and proteins found within the body. The immune system is designed to destroy invading foreign particles by producing antibodies that recognise proteins on the invading pathogen. However, if a foreign protein is sufficiently similar in structure to a protein or proteins within the body, the antibodies falsely recognise the body's own tissue as pathogenic, and the immune response damages both the foreign protein, and inadvertently, the body's own tissue. Professor Ebringer outlined examples of disease involving autoimmunity including rheumatic fever, ankylosing spondylitis and rheumatoid arthritis.
- 5.3 The possibility that BSE may be an autoimmune disease was first considered following the observation that mice with a deficient immune system appeared to be resistant to scrapie when inoculated peripherally. Under the prion theory, this was explained by the need for prion protein to replicate within the immune system prior to migration to the central

nervous system. However an alternative explanation was that if the immune system was required to induce BSE, it was possible that BSE could also be an autoimmune disease.

- 5.4 Professor Ebringer noted that antibodies against myelin, a central protein constituent of white matter in the brain, were responsible for the spongiform pathology associated with chronic experimental allergic encephalomyelitis (EAE) in mice. This was an autoimmune disease that was used as an animal model for multiple sclerosis (MS). In view of the related spongiform pathology in both EAE and BSE, Professor Ebringer investigated if any bacterial proteins had similar sequences to bovine myelin. Database searches revealed that bovine myelin contained a similar structural sequence to a peptide found in *Acinetobacter*, a common environmental bacterium. Professor Ebringer proposed that the ingestion of *Acinetobacter* bacteria would induce an immune response in cattle. This would in turn produce antibodies which would attack both the invading pathogen, and because of molecular mimicry, the myelin in brain tissue. Professor Ebringer hypothesised that this causes the neurological condition known as BSE.
- 5.5 Professor Ebringer summarised published work funded by MAFF which showed significantly elevated levels of *Acinetobacter* antibodies in the blood of BSE positive cattle in comparison to controls. No such elevations were seen for antibodies for six other common bacteria (see ref. 2). Professor Ebringer also reported that the highest antibody levels were from the IgA antibody isotype associated with immunity from the gut. He considered that this gave a strong indication that the offending antibodies were generated primarily from the intestine, which implicated the ingestion of *Acinetobacter* bacteria or bacterial fragments in cattle feed, rather than a secondary immune response following prion-induced damage to the brain.
- 5.6 Professor Ebringer outlined the technology and preliminary results of an ante-mortem diagnostic test that he had developed based on the observation of elevated antibody levels. The M.A.N. index measures three antibodies; myelin autoantibodies, *Acinetobacter* antibodies and Neurofilament autoantibodies (i.e. antibodies produced by the body in response to *Acinetobacter* that bind to neural tissue). The index was used to study blood from 403 cattle, 189 of which were cases of BSE. Professor Ebringer indicated that the mean MAN index showed a significant elevation of anti-*Acinetobacter* antibodies in comparison to controls, and that 70% of BSE positive animals were above the 95% confidence limits of the controls.
- 5.7 During subsequent discussion, Members agreed that it was likely that markers such as elevated antibody levels may be an important element

in developing a diagnostic test for the rapid detection of BSE. However Members considered that evidence of elevated anti-myelin and anti-neurofilament antibody levels during the incubation period for BSE would be firmer evidence that the immune response to the bacteria was a possible primary cause of neuronal damage rather than a non-specific secondary effect associated with cell death.

- 5.8 Members also considered the evidence cited by Professor Ebringer that immuno-deficient mice were not susceptible to scrapie. It was noted by Professor Ebringer's team that the primitive immune system still remained intact in these mice. However, SEAC members noted that studies had shown that when immunodeficient mice are inoculated directly into their brain rather than peripherally, they do contract TSE disease. It was also noted that up to 30% of immunodeficient mice contract TSE infection when inoculated peripherally. Overall this gave a strong indication that although the immune system may be involved in the initial replication of the disease agent following peripheral exposure, a functional immune system was not required for the development of BSE infection.
- 5.9 Members also noted that the central role of the prion protein in BSE aetiology has been widely demonstrated. An example includes the dramatic effect on disease susceptibility following the alteration of a single amino acid within the prion protein of transgenic mice which are otherwise identical. Members were concerned that Professor Ebringer had a very limited view of how his theory fitted with the extremely large body of other scientific research and evidence in the field, much of which led to very different conclusions on the cause of these diseases.
- 5.10 Members expressed concern that the preliminary data showing elevated antibody levels in BSE affected cattle did not take sufficient account of other factors that could have effected the results. This applied both to the data on elevated *Acinetobacter* levels and the results of the MAN assay. For example, the data did not take adequate account of the age, breed or geographical location of the BSE affected animals used in the studies. Given that BSE generally occurs in older animals, from the presented data it was not possible, for example, to rule out the possibility that the difference between the BSE infected animals and the controls described by Professor Ebringer was due, at least in part, to the BSE-infected animals being older, on average, than the controls, and older animals having a higher level of *Acinetobacter* antibodies.
- 5.11 In regard to the association between vCJD and BSE, Professor Ebringer indicated that in his view no direct evidence had been produced that the consumption of infected meat was linked to the appearance of vCJD. He noted a recent publication from Dr Venters ([see ref. 4](#)) suggesting that

the vCJD was not a new disease, but had been identified because of heightened awareness following the discovery of BSE. Members again considered that this view ran counter to the large body of biochemical and epidemiological evidence linking vCJD and BSE, including the recent conclusions from the investigation of the geographically associated cases in Leicestershire which implicated butchery practices as a possible cause. It was also reported that the characteristic spongiform pathology seen in vCJD was unique, and affected neuronal tissue (grey matter) in the brain. This was very different from the spongiform degeneration associated with the autoimmune disease EAE cited by Professor Ebringer, which primarily affects the white matter (myelin) within the brain.

- 5.12 Professor Ebringer considered that the initial epidemic of BSE was unique to the UK because changes in rendering practice increased the levels of bacteria, or bacterial fragments entering cattle feed. He noted that he had requested samples of rendered material in order to test for the presence of *Acinetobacter*, but these had not been forthcoming. However Members noted that environments suitable for *Acinetobacter* bacteria are commonly found throughout the world and equivalent rendering practices were also employed worldwide. Members agreed that the hypothesis proposed by Professor Ebringer did not adequately explain much of the epidemiological data, including why the BSE epidemic primarily affected the UK, was found only in bovines, and only occurred in the 1980s.
- 5.13 Overall the Committee considered that Professor Ebringer supported his theory by using a rather selective and limited choice of publications, and had disregarded much of the published literature. They concluded that the theory was not a good postulate for the origin of BSE and that it did not seriously challenge the prion hypothesis.
- 5.14 The Committee also considered the possibility that antibodies to *Acinetobacter* might, despite the flaws in Professor Ebringer's theory, be a useful marker for an ante mortem test for BSE. Members agreed that, although the data presented on the M.A.N. assay was of some interest, a sensitivity of 70% was not sufficient to give confidence that BSE positive animals could be correctly identified. There was too much overlap between the antibody levels seen in individual control animals, and those seen in the BSE infected animals. Members also agreed that the specificity of the test also appeared to be poor compared to existing tests. The Committee concluded that on the basis of the results presented, there was little justification at this time for carrying out additional work to develop the test for use in pre-clinical animals.

References

1. Ebringer et.al. (1997) Bovine Spongiform Encephalopathy: Is it an Autoimmune Disease Due to Bacteria Showing Molecular Mimicry with Brain Antigens. Environ Health Perspect. **105**: 1172-1174.
2. Ebringer et al (1998) Bovine Spongiform Encephalopathy: Comparison between the 'Prion' Hypothesis and the Autoimmune Theory. J of Nutrit & Environ Med. **8**: 265-276
3. Tiwana et al (1999) Autoantibodies to Brain Components and Antibodies to *Acinetobacter calcoaceticus* Are Present in Bovine Spongiform Encephalopathy. Infection And Immunity. **67**: No. 12 : 6591-6595.
4. Venters (2001) New Variant Creutzfeldt-Jakob disease: the epidemic that never was. BMJ: **323** 858-861