



SEQUENCING *PRNP* OF BARB CASES

ISSUE

1. To inform the committee about two studies examining the DNA sequence of regions of *PRNP* (the gene that codes for the prion protein) of BARB cases and controls.

BACKGROUND

2. There have been around 100 BSE cases born since the UK reinforced mammalian meat and bone meal ban in August 1996. These BSE cases are referred to as BARB (born after the reinforced ban) cases. The cause of BARB cases is unclear. A number of hypotheses have been proposed to explain the cause, including a possible genetic predisposition to the disease as a result of polymorphism(s) in *PRNP*.
3. *PRNP* polymorphisms are a determinant of susceptibility to TSEs in other species. However, studies on a possible genetic predisposition to BSE in cattle born before the reinforced ban have failed to find strong associations between *PRNP* polymorphisms and susceptibility to BSE. A study of UK born after the 1988 ban (BAB) BSE cases (n= 18) and BSE-negative controls (n= 9) found no significant differences in *PRNP* sequence between the two groups (Defra project SE1716). A study of German cattle (n= 43 BSE cases and n= 48 BSE-negative controls) has shown a possible association between BSE susceptibility and sequence variation of the *PRNP* promoter region but not polymorphisms elsewhere on the gene¹.
4. To examine whether variations in the sequence of *PRNP* may be involved in the aetiology of BARB cases, two case-control studies have been set up to examine the sequence of specific *PRNP* regions: a study examining Great Britain (GB) BARB cases and a study of Northern Ireland (NI) BARB cases.

¹ Sander *et al.* (2004) Analysis of sequence variability of the bovine prion protein gene (*PRNP*) in German cattle breeds. *Neurogenetics* 5, 19-25

PREVIOUS SEAC CONSIDERATION OF BARB CASES

5. In November 2003, SEAC discussed the possible origins of BARB cases. The committee noted that the incidence of BARB cases was too high to support the hypothesis that a sporadic occurrence of the disease was solely responsible. Furthermore, BARB cases were geographically different and more randomly distributed compared with BSE cases born before the reinforced ban. Although the difference in geographical distribution between these two types of BSE case did not support an obvious environmental cause for BARB cases and was inconsistent with the distribution of contaminated feed from previous suspect sources, the committee considered it important to investigate the origins of feed materials and colostrum fed to BARB cases. The committee recommended genotyping, biochemical and strain typing studies be pursued, as well as an epidemiological case-control study on BARB cases, to address the issue of possible sources of infection.
6. In March 2004, an *ad hoc* SEAC Epidemiology Subgroup on UK BARB cases was convened to advise on the design of a case-control study to identify possible causes of BARB cases. The Subgroup was reconvened in April 2005 to discuss preliminary results from the study. The Subgroup recommended (endorsed by SEAC in April 2005) further analysis using different criteria to define the controls used in the study. In addition, it was recommended to continue the study using a more sophisticated group of controls and for a prospective evaluation of animal feed use and supply routes to assess the potential for cross-contamination of feeds.
7. In March 2005, the committee was updated about a Defra-funded project to sequence *PRNP* of BARB cases with control animals matched for breed and birth cohort. The committee were informed that preliminary analysis has not shown significant differences in *PRNP* sequence between the two groups. The committee welcomed such data and asked that the study be completed as rapidly as possible.

GB STUDY

8. A Defra funded project is investigating whether polymorphisms in the open reading frame and regulatory regions of *PRNP* in GB BARB cases may be associated with susceptibility to BSE. DNAs extracted from BARB cases (n= 64) and farm-matched healthy control animals (n= 65) have been sequenced. Preliminary analysis of the *PRNP* open reading frame has revealed no significant differences between the sequence of this region between BARB cases and controls. Further sequencing and analysis of the open reading frame and regulatory regions is ongoing. Further details of the study are given at Annex 1.

NI STUDY

9. A study by Veterinary Science Division, Department of Agriculture and Rural Development, Northern Ireland (DARDNI) has investigated polymorphisms in the promoter and coding regions of *PRNP* as well as the coding region of the prion Doppel gene (a prion-like protein encoded by a separate gene, *PRND*). These regions were sequenced from NI BARB cases (n= 13), BSE cases born before the reinforced ban (n= 13) and age matched negative controls (n= 26). Polymorphisms identified in these sequences showed no significant association between BSE cases born before and after the reinforced feed ban. However, significant differences were found within the *PRNP* promoter region between all the BSE cases (both born before and after the reinforced feed ban) and the negative controls. Results from this study will be presented at SEAC 88.

ADVICE SOUGHT FROM THE COMMITTEE

10. The committee is invited to comment on the research findings presented.

Research to investigate polymorphisms at *PRNP* in Born After the Reinforced Ban (BARB) cases of BSE

Summary

Defra has funded a research project at the Veterinary Laboratories Agency (VLA) to investigate if variation in the coding or regulatory regions of *PRNP* gene is present in the BARB cases of BSE. By the end of the project, approximately 6kb from around 85 BARB animals and 65 age-, breed and natal-herd-matched controls will be sequenced and examined to determine if specific polymorphisms or predicted haplotypes can be associated with the BARB population. Background information about the project, which is in progress and has a completion date of November 2005, is provided below. An interim report will be provided by the VLA Project Leader at SEAC 88.

Introduction

1. Epidemiological evidence and the effect of the 1988 and 1996 feed bans supports the belief that transmission of BSE in cattle occurred through contaminated mammalian meat and bone meal (MBM) in animal feed. However, the origin of BSE has not been ascertained and various hypotheses remain².
2. Around 100 BSE cases have been reported in cattle born after 1st August 1996 when MBM was banned from pig and poultry feed. These BSE cases are referred to as BARB (born after the reinforced ban) cases.
3. To date, the tissue histopathology and prion protein immunochemistry of BARB animals is identical to that observed in all other bovine BSE cases in the UK. BARB cases are geographically widespread and are found in a range of breeds. Professor John Wilesmith has suggested that these cases may have arisen through a residual, low level contamination of animal feed with infected MBM, possibly through cross-contamination of feed trans-shipped through European ports.
4. The origin(s) of the source of infection and the route(s) of transmission of BSE in BARB animals remain to be resolved. It is not known whether there is a single cause or whether there are multiple causes of the disease in these animals. The BARB cases may be particularly useful in investigating potential causes of BSE unrelated to contaminated feed.
5. If the origin of the disease/route of transmission cannot be identified for all BARB cases, and the number of BARB animals does not decrease year on year, it is unlikely that Defra will achieve its aim of eradicating BSE by 2010. Therefore, in addition to information collected through standard surveillance procedures, Defra is taking three approaches to investigate BARB cases:
 - The case-control study being overseen by the *ad hoc* Epidemiology subgroup on UK BARB cases

² See <http://defraweb/animalh/bse/publications/bseorigin.pdf> for the 2001 report of the committee that reviewed the state of understanding on the origin of BSE.

- An independent review of BARB cases being conducted by Professor Hill, University of Edinburgh
- A research project at the Veterinary Laboratories Agency (VLA) to investigate *PRNP* molecular genetics of BARB cases of BSE

6. This paper describes the research project and reports briefly on its status.

Rationale underpinning the research

7. If there is a genetic component in the aetiology of BARB cases, observations from other species suggest that the *PRNP* locus is likely to be involved. There are two main types of variation at *PRNP* that could theoretically contribute to the origin of BARB cases. These are:

- Rare inherited or *de novo* mutations that lead to an increased rate of generation of the disease-associated form of the prion protein in the absence of an infectious agent (analogous to familial CJD)
- Existing variants of *PRNP* that give rise to an increased susceptibility to extremely low levels of TSE agent present, for example, in the environment or in contaminated feed.

8. Professor Malcolm Ferguson-Smith, University of Cambridge has proposed previously that BARB cases might be “direct descendants of an original animal which carried the novel mutation that started the epidemic over 30 years ago”³. Since BARB cases are now known to be dispersed throughout the UK in unrelated herds and across different breeds (Table 1), Professor Ferguson-Smith’s suggestion is unlikely to provide the sole explanation of BSE in the BARB population but could theoretically explain the existence of several cases within a breed.

Table 1. Cattle breeds represented in the population of BARB cases

Pure breeds	Number of BARBs ^a	Cross breeds	Number of BARBs ^a
Friesian/Holstein	62	Limousin cross	8
Jersey	2	Simmental cross	6
Highland	2	Charolais cross	5
Aberdeen Angus	1	Holstein/Friesian cross	4
Devon	1	Aberdeen Angus cross	1
Guernsey	1	Belgian Blue cross	1
Limousin	1	Hereford cross	1
Meuse Rhine Isel	1	Salers cross	1
		Shorthorn cross	1
TOTAL	71	TOTAL	28

^a number of BARB cases of this breed type reported by April 6th 2005 (courtesy of Defra BSE Statistics and Science Branch)

³ “Continuing anxiety about BSE” in Veterinary Record 153, p723 (2003).

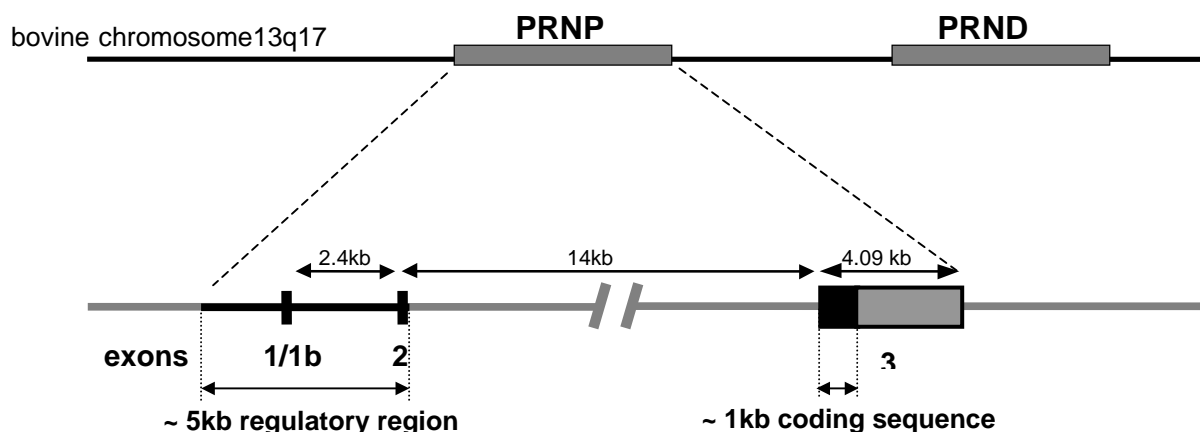
9. To provide evidence that might confirm or refute whether these possibilities might contribute to the aetiology of BSE in the BARB population, Defra has funded work to sequence the open reading frame (ORF) and regulatory regions of the prion protein gene in BARB cattle. Details of this project are provided below.

Research Project SE0239: Investigation of polymorphisms associated with BSE in BARB cases

Outline of project

10. This research project is a collaboration between Dr. Ginny Saunders and Dr. Peter Griffiths (VLA, Weybridge) and Dr. John Williams (Roslin Institute, Edinburgh). Following recommendations from the Defra TSE Research Advisory Group and independent peer reviewers' comments, funding was awarded to sequence the PRNP coding sequence and regulatory regions in the 78 BARB cases identified at the start of the project and one farm-, breed and age-matched control per BARB (see Figure 1). The project started in May 2004.

Figure 1. Regions of PRNP sequenced in Project SE0239



11. It is recognised that additional controls might be required to investigate the within-breed frequency of any interesting polymorphisms but such work cannot be planned until the sequencing data has been analysed and reviewed.
12. Individual polymorphisms and predicted haplotypes identified from the sequence data will be analysed to examine whether there is any association with BARB status. Further research might include targeted analysis of any polymorphisms associated strongly with BSE susceptibility in 500 BSE positive and 500 clinically normal samples collected in the mid-1990s.
13. The PRNP genes in additional BARB cases reported before March 2005, including the case born in 2001, will also be sequenced and will be included in

the overall statistical analysis. For any new BARB cases identified between April 2005 and the end of the project in November 2005, only the PRNP open reading frame will be sequenced, unless developments indicate that a more extensive analysis is required.

Current status of research work

14. Initially, material was obtained from 77 BARB animals in the form of blood, brain tissue or brain homogenates. For 65 of the 77 BARBs, two farm-, breed-, age- and sex-matched control animals were available and blood samples were sourced through the State Veterinary Service. Due to the high cost of sequencing, only one control is being sequenced for each BARB. The collection of samples from the controls was not completed until January 2005.
15. At the end of April 2005, the *PRNP* open reading frame had been sequenced in 64 BARB animals and 65 matched controls. Because of the poor quality of some of the samples, incomplete or reduced quality sequence is available for 9 samples and no DNA data could be obtained for a further 4 samples.
16. Sequencing of the regulatory regions from these samples is in progress.
17. Preliminary analysis of the *PRNP* open reading frame has revealed no significant differences between the BARB cases and controls as reported at SEAC 87. A more detailed statistical analysis is underway and will be reported at SEAC 88.

Studies on BARB cases in Northern Ireland

18. A similar sequencing study has been undertaken by DARDNI on the BARB cases reported in Northern Ireland. In this study BARB cases (13), BSE cases reported before 1996 (13) and controls (26) were sequenced in slightly different regions to those of the GB study. The regions studied included ~1.4kb of the *PRNP* promoter region, the PrP coding sequence and the PrP Doppel (*PRND*) coding sequence.
19. Polymorphisms identified during the course of these studies showed no significant association with either class of BSE cases. Full details will be presented at SEAC 88.